MEDICAL LIBRARY

APR 25 1949

THE JOURNAL OF CLINICAL ENDOCRINOLOGY

VOLUME 9 NUMBER 3

Next Annual Meeting, Haddon Hall, Atlantic City, June 3 and 4, 1949

MARCH 1949

ANTIHORMONE FORMATION COMPLICATING PITUITARY GONADOTROPIN THERAPY IN INFERTILE MEN. I. PROPERTIES OF THE ANTIHORMONES 213
William O. Maddock

THE PREGNANDIOL PRECIPITATION TEST. FURTHER OBSERVATIONS ON CLINICAL APPLICATIONS AND TECHNIC 234
Harold C. Mack, Arthur E. Parks and Marian McDonald

CARBOHYDRATE METABOLISM IN THE COMBINATION OF DIABETES MELLITUS AND ADDISON'S DISEASE, AS ILLUSTRATED BY A CASE . . 245
Joseph H. Crampton, Sidney T. Scudder and Clarence D. Davis

CORTICOADRENAL TUMOR WITH HYPOGLYCEMIC SYNDROME, GOITER, GYNECOMASTIA AND HEPATOSPLENOMEGALY 255
Juan José Staffieri, Oscar Cames and José M. Cid

THE URINARY EXCRETION OF CHORIONIC GONADOTROPIN BY HUMAN FEMALES FOLLOWING PARENTERAL ADMINISTRATION OF AQUEOUS OR BEESWAX SOLUTIONS 268
C. W. Lloyd, E. C. Hughes, M. L. Eva and J. Lobotsky

RESULTS OF PROLONGED MEDICAL TREATMENT OF OBESITY WITH DIET ALONE, DIET AND THYROID PREPARATIONS, AND DIET AND AMPHETAMINE 275
David Adlersberg and Martin E. Mayer

UTERO-TUBAL PERSUFFLATION CURVE IN MYXEDEMA. EFFECT OF THYROID THERAPY 285
J. C. Mussio Fournier and A. Pou de Santiago

THE 1949 MEETING OF THE ASSOCIATION FOR THE STUDY OF INTERNAL SECRETIONS 292

THE JEFFERSON MEDICAL COLLEGE AND HOSPITAL FELLOWSHIP . . . 292

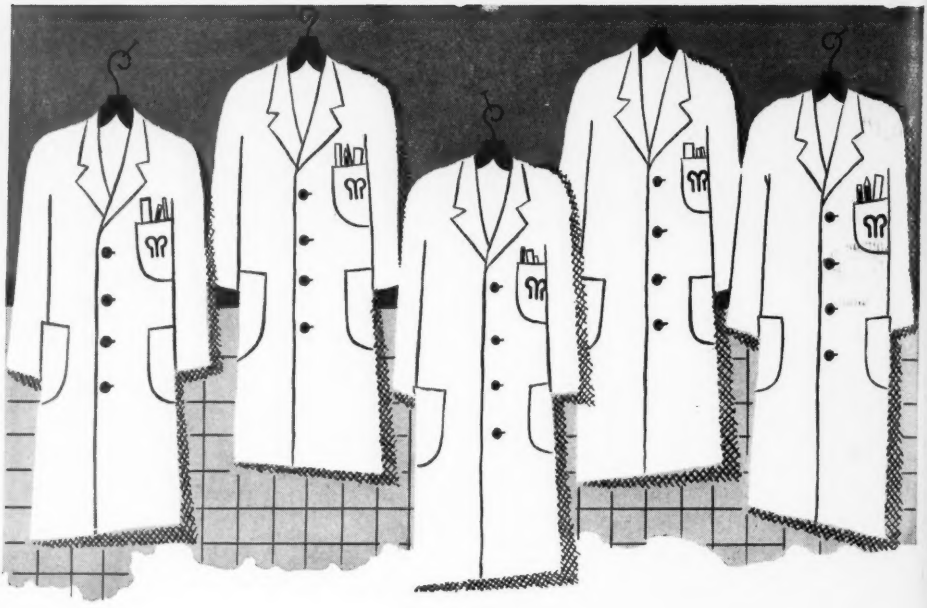
PROGRAM OF THE 1949 MEETING OF THE AMERICAN GOITER ASSOCIATION 293

COURSE IN MEDICAL ILLUSTRATION, UNIVERSITY OF GEORGIA 295

THE 1949 MEETING OF THE AMERICAN DIABETES ASSOCIATION . . . 296

ABSTRACTS OF CURRENT ENDOCRINE LITERATURE 297

Issued monthly for The Association for the Study of Internal Secretions by Charles C Thomas, Publisher. Annual subscription, $7.50; single copies, $1.00



The mantles of the men who want a better thyroid

They seek better thyroid therapy and so they offer PROLOID

1. Extracted from fresh thyroid material and carefully assayed for potency so that the different thyroid batches can be balanced and then blended for more uniform ultimate potencies.

2. So purified of unwanted organic matter as to be free from the tell-tale odor of ordinary thyroid.

3. Standardized *metabolically* as well as chemically to guard the patient against metabolic fluctuations.

Available in ¼, ½, 1, 1½ and 5 grain tablets—bottles of 100, 500 and 5,000. Also as powder—1 oz. and 8 oz.

Proloid THE IMPROVED THYROID MEDICATION

The Maltine Company

SERVING THE MEDICAL PROFESSION SINCE 1875 • MORRIS PLAINS, NEW JERSEY

THE JOURNAL OF
CLINICAL
ENDOCRINOLOGY

VOLUME 9 MARCH, 1949 NUMBER 3
Copyright 1949 by the Association for the Study of Internal Secretions

ANTIHORMONE FORMATION COMPLICATING PITUITARY GONADOTROPIN THERAPY IN INFERTILE MEN

I. PROPERTIES OF THE ANTIHORMONES*

WILLIAM O. MADDOCK, M.D., PH.D.**

Department of Physiology, University of Oregon Medical School, Portland Oregon

PROLONGED administration of gonadotropic hormones derived from one species to an individual of a different species can elicit formation of substances in the plasma capable of preventing the action of the administered hormones. These substances have been named *antihormones*, or more specifically, *antigonadotropins*. Such terminology infers a similarity between antihormone and antibody formation; however, it is not the purpose of this report to consider the immunological properties of antihormones. This subject has been amply reviewed by Zondek and Sulman (1).

Antigonadotropins, besides being effective against the administered hormone, may also be effective against other gonadotropic hormones. In experimental animals, it has been demonstrated that nonspecific antigonadotropins may even be effective against the normal, circulating gonadotropins. For example, Rowlands (2) found that nonspecific antihormones were formed in the serum of rabbits injected with an ox anterior pituitary ex-

Received for publication July 12, 1948.

*Read in part before the 57th Annual Meeting of the American Physiological Society, Atlantic City, New Jersey.

Supported in part by a grant from the Research Grants Division of the U. S. Public Health Service.

**Eli Lilly Fellow in Endocrinology.

tract. Administration of this serum to male or female rats was followed by atrophy of the gonads, similar to that occurring after hypophysectomy.

The problem of antihormone formation is therefore of considerable importance in clinical therapy with gonadotropic hormones. Besides vitiating the effects of the administered hormone, the formation of nonspecific antihormones may also nullify the actions of the patient's endogenous gonadotropic hormones. This possibility has been recognized, but as yet has not been demonstrated (3).

Antihormone formation in human subjects has followed administration of pregnant mare serum gonadotropin (4, 5, 6), horse pituitary gonadotropin (7), and a mixture of human chorionic gonadotropin and sheep pituitary gonadotropin ("Synapoidin") (6). The antihormones formed to pregnant mare serum were specific (4, 6), *i.e.*, they were effective only against pregnant mare serum gonadotropin. The antihormones formed to "Synapoidin" and to horse pituitary gonadotropin were nonspecific, as they were effective against pregnant mare serum as well as against the administered hormones (6, 7). There was no evidence that the antihormones were effective against the patients' endogenous gonadotropic hormones (6).

It is the purpose of this communication to report the results of studying antigonadotropin formation in 7 sterile men receiving a sheep anterior pituitary extract rich in follicle-stimulating hormone[1] (hereafter referred to as sheep FSH). The following problems were investigated:

1. Rate, incidence and amount of antihormone formation.
2. Specificity of the antihormones.
3. Effects of the antihormones on the patients' endogenous gonadotropins.
4. Mechanism of action of the antihormones.

Method of antihormone assay: Twenty-four-day old female Sprague-Dawley rats were used as assay animals. A sample assay is presented in Table 1. Each rat received a total of 4 units[2] of sheep FSH dissolved in 6.0 cc. of water, 1.0 cc. being injected subcutaneously twice daily for three days. One group of rats received only FSH, whereas others received in addition varying amounts of plasma suspected of containing antihormones (obtained from patient K.H. eighty-two days after initiating therapy). The plasma was divided into 6 equal doses and injected subcutaneously concurrently with the hormone, but at a separate site. The rats were killed

---

[1] Generously supplied by the Schering Corporation through the courtesy of Dr. Edward S. Henderson. (This preparation also contained small amounts of interstitial cell-stimulating hormone.)

[2] "Units" refers to the manufacturers' stated potency. This also applies to the other hormones used.

twenty-four hours after the last injection, and the weights of ovaries and fluid-free uteri were taken as the assay end points. (Controls receiving only FSH were included in each assay, because it was found that the amount of hormone varied from ampule to ampule. It should be stressed, however, that in each assay all rats received exactly the same amount of hormone from the same stock solution, freshly prepared prior to each assay.)

The data of Table 1 demonstrate that rats receiving only FSH showed marked uterine and ovarian stimulation. Rats receiving in addition 0.9 cc. of plasma showed only uterine stimulation, whereas rats receiving 1.8 cc. of plasma showed neither ovarian nor uterine stimulation.

TABLE 1. DETECTION OF ANTIGONADOTROPINS

Units of sheep FSH per rat*	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
4	0	109	83	3
4	0.9†	146	15	2
4	1.8†	37	13	3
Uninjected controls‡	0	36	13	63

* The FSH and plasma were divided into 6 equal doses administered twice daily for 3 days.

† Source of plasma: K.H., 82 days following initial FSH injection.

‡ These values for uninjected controls were obtained from controls included in each assay and apply to all the data.

Certain details concerning the response of the immature female rat to gonadotropic hormones should be emphasized. The first response of the ovary to gonadotropic hormones is secretion of estrogen, which is detected by an increase in weight of the uterus. Only after the uterine weight increase has reached a maximum, does the ovary increase in weight (8). The importance of this concept is illustrated by inspection of the data in Table 1. Compare first the data for rats receiving only FSH with those for rats receiving in addition 0.9 cc. of plasma. If only uterine weight is considered, no difference between the two groups can be detected, because both 109 and 146 mg. can be considered as approximately maximal uterine weights (8). However, a clear-cut difference is found by comparing ovarian weights, 83 *vs.* 15 mg. Similarly, compare the figures for rats receiving 0.9 cc. of plasma with those of rats receiving 1.8 cc. of plasma. Ovarian weights show

no detectable difference, as both are at uninjected control levels. However, uterine weights now show a clear-cut difference, 146 *vs.* 37 mg.

The individual assay results for each patient are tabulated in Tables A to G in the appendix, and summarized in Table 2.

TABLE 2. RATE AND AMOUNT OF ANTIGONADOTROPIN FORMATION TO SHEEP FSH

Patient	Detection of anti-hormones before therapy	Duration of therapy (days) (50 units sheep FSH daily)	Anti-hormones first detected (days)	Maximal amount (units of anti-hormone* in circulation)	Last day found present (after stopping therapy)	First day found absent (after stopping therapy)
D.B.	None	65	52	3,000	37	86
H.B.	None	104	51	5,000	39	88
R.C.	None	84	60	>7,000	60	125
L.D.	None	82	49	10,000	58	108
C.G.	—	60	56	>7,000	283	—
K.H.	None	66	52	10,000	128	164
G.M.	—	56	45	3,000	21	121
Range	None (5 tested)	56–104	45–60	3,000–10,000	21–283	86–

* One "antihormone unit" is that amount just sufficient to prevent the action of 1 unit of sheep FSH.

RATE, INCIDENCE AND AMOUNT OF ANTIHORMONE FORMATION

Antihormone titers before therapy: Antihormone titers were determined in 5 of the 7 patients before initiating therapy by assaying 0.9 cc. of plasma against 2 units of sheep FSH. In 2 normal students 6.0 cc. of plasma was assayed against 4 units of FSH (Table H, appendix). In no instance were antihormones detected in untreated individuals.

Duration and amount of therapy: Each patient received 50 units of sheep FSH daily (self-administered, intramuscularly) except patients K.H. and G.M. Patient K.H. received 50 units twice daily for twelve days and then 50 units once daily for fifty-four days. Patient G.M. received 50 units twice daily for forty-five days and then 50 units once daily for eleven days. Duration of therapy ranged from 56 to 104 days, and is indicated for each patient in Table 2. Pretreatment intradermal sensitivity tests to the sheep FSH were negative in each patient.

Incidence of antihormone formation: Antihormone formation occurred in each of the 7 patients. By determining antihormone titers at approximately two to four week intervals it was found that all 7 patients developed antihormones within forty-five to sixty days after initiating therapy (Table

2). At this time, antihormone titers were sufficiently great to nullify many times the administered daily dose of sheep FSH.

Amounts of antihormone present in the circulation: The amounts of antihormone present in the circulation can be roughly estimated as follows: from Table 1, it can be seen that 0.9 cc. of K.H.'s plasma almost, but not completely, prevented the action of 4 units of FSH It can be estimated that approximately 3 units of FSH were inactivated. Defining an "antihormone unit" as that amount just sufficient to prevent the action of 1 unit of hormone, then 0.9 cc. of K.H.'s plasma contained about 3 units of antihormone. Assuming total plasma volume as 3,000 cc., then there were $3{,}000/0.9 \times 3$, or 10,000 units of antihormone in the total plasma. This is enough to neutralize 200 times the administered daily dose, or almost 3 times the total amount of hormone administered. Similar estimations for the other patients showed that at the time of maximal antihormone formation there were 3,000 to 10,000 units of antihormone present in the plasma (Table 2).

Time required for antihormones to disappear: Repeated antihormone assays performed after stopping therapy demonstrated that antihormones disappeared from the plasma within three to five and one-half months after the last injection of FSH in 6 of the 7 patients (Table 2). At the date of the last test, 6.0 cc. of plasma of each of these patients did not alter the effects of 4 units of sheep FSH. Antihormones were still present in the other patient 283 days after cessation of therapy; at this time the titer was approximately one-fourth of the maximal value.

SPECIFICITY OF THE ANTIHORMONES

In order to test the specificity of the antihormones, plasma containing antihormones to sheep FSH was tested against the following gonadotropic hormones:

1. Chorionic gonadotropin derived from human pregnancy urine.
2. Pregnant mare serum gonadotropin.
3. Anterior pituitary gonadotropin derived from horse pituitaries (mainly FSH).
4. Urinary gonadotropin derived from the urine of a castrated man (mainly FSH).

The assays were carried out as previously described.

Antigonadotropic effect of plasma on human chorionic gonadotropin: Two commercial preparations were tested: "Pranturon" (Schering) and "A.P.L." (Ayerst, McKenna & Harrison). In both instances, 1 unit of hormone elicited a definite gonadotropic response which was completely prevented by the injection of 1.8 and 3.0 cc. of plasma of patients L.D. and C.G., respectively (Table 3).

TABLE 3. ANTIGONADOTROPIC EFFECT ON HUMAN CHORIONIC GONADOTROPIN

Units of chorionic gonadotropin per rat	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
"Pranturon" (Schering)				
1	0	91	22	3
1	1.8*	29	9	3
"A.P.L." (Ayerst)				
1	0	125	19	3
1	3.0†	45	13	3

Source of plasma: * L.D., 105 days following initial FSH injection.
† C.G., 264 days following initial FSH injection.

Antigonadotropic effect of plasma on pregnant mare serum gonadotropin: Three cc. of patient C.G.'s plasma completely prevented the action of 5 units of pregnant mare serum ("Antex," Ayerst, McKenna & Harrison) (Table 4).

TABLE 4. ANTIGONADOTROPIC EFFECT ON PREGNANT MARE SERUM (PMS)

Units of PMS ("Antex," Ayerst) per rat	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
5	0	135	15	3
5	3.0*	41	15	3

* Source of plasma: C.G., 265 days following initial FSH injection.

Antigonadotropic effect of plasma on horse FSH: The antihormones failed to alter the effect of 1 unit of horse pituitary gonadotropin ("Gonatrope," Forbes). Although 1.8 cc. of patient D.B.'s plasma collected sixty-six days after initiating FSH therapy was capable of preventing the response of about 2 units of sheep FSH, the same amount of this plasma failed to affect the response of 1 unit of horse pituitary gonadotropin. Similarly, 3.0 cc. of patient C.G.'s plasma collected 264 days after initiating therapy prevented the effect of approximately 2 units of sheep FSH; but 3.0 and 6.0 cc. of this plasma were without effect on 1 unit of the horse pituitary

preparation (Table 5). Thus, plasma containing antihormones formed in response to sheep FSH was not antigonadotropic to horse pituitary gonadotropin.

TABLE 5. ANTIGONADOTROPIC EFFECT ON HORSE ANTERIOR PITUITARY EXTRACT

Units of horse FSH ("Gonatrope," Forbes) per rat	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
1	0	150	20	9
1	1.8*	120	18	3
1	3.0†	116	20	3
1	6.0†	138	24	3

Source of plasma: * D.B., 66 days following initial FSH injection.
† C.G., 264 days following initial FSH injection.

Urinary gonadotropin obtained from a castrated man: The urine was concentrated by the ultrafiltration technic (9). A two-hour aliquot gave a definite gonadotropic response which was partially prevented by 0.9 cc. of plasma and completely prevented by 6.0 cc. of plasma (collected from patient K.H. sixty-seven days after initiating therapy) (Table 6).

TABLE 6. ANTIGONADOTROPIC EFFECT ON HUMAN URINARY GONADOTROPIN

Aliquot hours; human male castrate ultrafilter urine concentrate per rat	Volume of plasma injected cc.	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
2	0	114	55	3
2	0.9*	117	32	2
2	6.0*	21	5	3

* Source of plasma: K.H., 67 days following initial FSH injection.

Antihormones that form in response to administration of sheep FSH are effective against hormones from sheep, horse, and human sources, and therefore *are not species specific.* The antihormones are effective against anterior pituitary, human chorionic, and pregnant mare serum hormones, and therefore *are not hormone specific.* However, this lack of specificity is not complete, because the antihormones are not effective against horse pituitary gonadotropin.

EFFECTS OF THE ANTIHORMONES ON THE PATIENTS' ENDOGENOUS GONADOTROPINS

Since the antihormones were sufficiently nonspecific to be active against human castrate male gonadotropins, the question arose whether or not they were effective against the patients' own urinary gonadotropins. Antihormones were tested against endogenous gonadotropins in 4 of the patients. Urine collected after stopping therapy (in order to avoid recovering

TABLE 7. EFFECT OF ANTIHORMONES ON PATIENTS' ENDOGENOUS GONADOTROPINS

Amount ultrafilter urine concentrate per rat	Total cc. plasma per rat	Days after therapy initiated, plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
12-hour urine per rat collected by R.C., days 54 to 63 after stopping therapy	0	—	86	12	3
	6.0	144	33	12	3
12-hour urine per rat collected by L.D., days 2 to 7 after stopping therapy	0	—	173	22	3
	3.0	91	28	12	3
12-hour urine per rat collected by K.H., days 6 to 9 after stopping therapy	0	—	113	12	2
	6.0	95	26	11	2
12-hour urine per rat collected by C.G., days 4 to 7 after stopping therapy	0	—	135	16	2
	0.9	77	28	11	2
4-hour urine per rat collected by C.G., days 126 to 131 after stopping therapy	0	—	82	14	4
	6.0	193	33	14	2

administered sheep FSH) was concentrated by ultrafiltration. Each rat in a given assay received an equal aliquot of the ultrafiltered urine concentrate. One group received only urine concentrate, whereas others received, in addition, plasma from the same patient. For example, patient L.D. collected urine from day 2 to day 7 after stopping therapy. The hormone concentrated from a twelve-hour urine specimen elicited definite uterine and ovarian stimulation; this was completely prevented by the addition of 3.0 cc. of his own plasma (Table 7). Similar results were obtained on the other 3 patients (Table 7).

The following control experiments were also performed: 6.0 cc. samples of plasma from normal medical students R.B., L.C., and E.J. were tested against twelve-hour urine extracts from patients C.G., K.H. and L.D. respectively, without materially effecting the gonadotropic response of the extracts (Table H, appendix). Also, the plasma of normal subject E J. was not antigonadotropic to his own urine extracts (Table H, appendix).

MECHANISM OF ACTION OF THE ANTIHORMONES

Although the antihormones are effective against endogenous gonadotropic hormones, gonadotropins are being excreted in the urine at a time when the antihormones in the blood are at their highest level (compare the time of urine collections (Table 7) with antihormone assays tabulated in the appendix). Also, urinary gonadotropin titers were as high or higher than pretreatment levels.[1] Therefore, antihormones do not destroy or irreversibly combine with gonadotropins nor do they prevent production or release of gonadotropic hormones from the hypophysis. As gonadotropic potency is clearly demonstrable in the urine extracts when antihormones are present in the plasma, separation of antihormone and hormone must occur. There are two possible sites where this could take place:

1. The kidney. The kidney could retain antihormone, but excrete hormone.
2. The ultrafilter concentration procedure. It is possible that the kidney excretes both hormone and antihormone and that ultrafiltration recovers *only* hormone from the urine.

To differentiate between these two possibilities, plasma known to contain antihormones was diluted 1:100 with water and then concentrated by the usual ultrafiltration technic. The concentrate was then extracted with water to the original volume of plasma and its antihormone content compared with that of the original plasma by assaying against sheep FSH. The data of Table 8 show that the ultrafiltration technic *is* capable of retaining antihormones, and that approximately 50 per cent of the original potency is recovered. Thus, the second possibility is not in operation, and separation of antihormones and hormones must take place at the kidney.

DISCUSSION

Rate and incidence of antihormone formation: Clinical data concerning the formation of antigonadotropins to pituitary extracts are meager. Meyer

[1] *See* Table 1 in part II of this study (Jungck, E. C.; Maddock, W. O.; Heller, C. G., and Nelson W. O.) to follow in the April issue of THE JOURNAL OF CLINICAL ENDOCRINOLOGY.

and Wolfe (10), referring to the unpublished data of Meyer and Sevringhaus, stated that antigonadotropins were formed in the blood of human females after the administration of anterior pituitary gonadotropin preparations; no further information was given. Spence, Scowen and Rowlands (11) were unable to detect antihormones in the serum of 2 patients following treatment with 30 to 50 units of a pig pituitary extract twice weekly for sixteen to twenty-three weeks. Leathem and Rakoff (7) reported that 6 of 13 patients treated with 200 to 400 units of horse pituitary gonadotropin per month developed antihormones after three to four months of therapy.

To our knowledge there are no previous data concerning the formation

TABLE 8. RECOVERY OF ANTIHORMONES FROM PLASMA BY ULTRAFILTRATION

Units sheep FSH per rat	Total cc. plasma per rat	Treatment of plasma	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
4	0	—	120	52	3
4	1.8*	untreated	97	15	3
4	3.0*	untreated	28	12	3
4	3.0*	ultrafiltration	80	17	3
4	0	—	101	77	3
4	0.9†	untreated	118	31	2
4	1.8†	ultrafiltration	118	29	3

Source of plasma: * C.G., 243 days following initial FSH injection.
† K.H., 109 days following initial FSH injection.

of antigonadotropins in the human following the administration of only sheep pituitary extracts. However, Rakoff and Leathem (12) reported the results of treating 25 patients with a mixture of sheep pituitary and human chorionic gonadotropins ("Synapoidin"). Antihormones did not form in 22 patients treated for two to five months. Antihormones did form in 3 patients treated for more than six months with this preparation. The formation of antihormones in their experiments is most probably due to the sheep FSH in the extracts, since antihormone formation has not been demonstrated following treatment with human chorionic gonadotropin alone. (Segaloff and Parson (13) have reported the presence of antihormones effective against human chorionic gonadotropin after the administration of this material. However, as antihormone assays were not performed prior to treatment with chorionic gonadotropin, and as this patient had previously received protracted treatment with a pituitary extract, it is most

likely that these antihormones were formed in response to the pituitary extract—not the chorionic gonadotropin.)

The low incidence of antihormone formation encountered by Rakoff and Leathem (12) compared to the 100 per cent incidence found by us may be explained on the basis of the difference in dosage employed. Whereas we employed 50 units of sheep FSH daily, they used only 90 units of "Synapoidin" monthly ("15 rat synergy units . . . 3 times weekly for the first 2 weeks of each cycle").

The lack of specificity of the antihormones formed in response to sheep FSH is in agreement with animal experiments (14, 15). The fact that the antihormones were not effective against horse pituitary gonadotropin is puzzling, but not without precedent. Simmonet and Michel (16) found that antihormones formed in rabbits following administration of human chorionic, pregnant mare serum, and postmenopausal urine gonadotropins were not effective against horse pituitary extracts. The only antihormone effective against horse pituitary gonadotropin was that formed in response to horse pituitary extracts.

Our data demonstrate that antihormones do not destroy, irreversibly combine with or prevent pituitary production or release of gonadotropins. Two alternate mechanisms of action may be considered:

1. The antihormones have no effect on gonadotropins, but act directly on the gonads, rendering them incapable of responding to gonadotropins.
2. Antihormone and hormone combine, in which form the hormone is incapable of acting on the gonads. Such a combination could, by definition, be separated by the kidney.

The first mechanism of action seems highly improbable. It is difficult to visualize how an antihormone could render the gonads incapable of responding to one hormone but not another, unless the mechanism of gonadotropic action varies as to the hormone employed. Okkels (17) has shown that thyroid tissue, unresponsive to the action of thyrotropin, again becomes responsive to the same thyrotropin when the thyroid gland is removed and placed in a perfusion apparatus. Selye, Collip and Thomson (18, 19) have demonstrated that rats refractory to one gonadotropic hormone are still capable of responding to other gonadotropins.

The second mechanism of action adequately explains the data. Whether or not an antihormone was effective against a given hormone would depend upon the ability of the two to combine. Such a theory explains the action of antihormones without assuming that they destroy hormones or act directly on the gonads.

Zondek and Sulman (20) demonstrated that antigonadotropins do not destroy gonadotropins in vitro. They treated a mixture of equal amounts

of hormone and antihormone with N/10 to N/15 NaOH and found that the antihormone was destroyed, leaving active gonadotropin.

Kupperman, Meyer and Hertz (21, 22) have concluded from experiments in parabiotic rats that antihormones do not act on the pituitary gland, but that they "neutralize" gonadotropins in the blood stream. We would agree with their conclusions and define "neutralization" as the formation of a combination of gonadotropin and antihormone.

That antihormones are not excreted in the urine of men with high plasma antihormone titers agrees with work in experimental animals (23, 24),

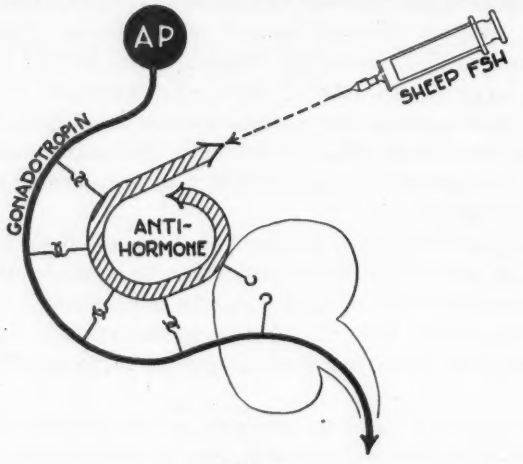


FIG. 1

where antihormones were not detected in the urine of animals having high titers of antihormones in the plasma.

The relationships between antihormones and endogenous gonadotropins are summarized diagrammatically in Figure 1. Administration of sheep FSH elicits the formation of nonspecific antihormones that are effective against the patients' endogenous gonadotropins. Antihormones act by combining with gonadotropins in the blood stream. The antihormone-hormone combination is separated by the kidney, antihormone being retained in the circulation and hormone being excreted in the urine.

SUMMARY AND CONCLUSIONS

1. In each of 7 sterile men given daily injections of follicle-stimulating hormone (FSH) derived from sheep anterior pituitary glands, antihormone formation occurred.

2. Sufficient antihormones formed within two months to vitiate completely the effects of the injected FSH.

3. The maximal titer of antihormones, attained within three months after initiating therapy, was sufficient to neutralize the effect of from 50 to 200 times the daily injected dose of FSH.

4. Antihormones disappeared from the circulation within three to nine months after stopping therapy.

5. Inasmuch as the antihormones were effective against gonadotropins derived from sheep, horse and human sources, they are not species specific; and inasmuch as they were effective against anterior pituitary, chorionic and pregnant mare serum gonadotropins, they are not hormone specific.

6. The antihormones found in the patients' plasma were capable not only of inactivating urinary gonadotropins derived from a castrated man, but also were able to prevent the action of the urinary gonadotropins of their hosts. It is therefore concluded that antihormones can prevent the actions of endogenous gonadotropins.

7. The mechanism whereby antihormones prevent the actions of gonadotropic hormones is apparently a reversible combination between the molecules of hormone and the molecules of antihormone.

It seems unlikely that antigonadotropins destroy gonadotropins or irreversibly combine with them, because endogenous gonadotropic hormones are being excreted in the urine when maximal amounts of antihormone are present in the plasma. It is concluded that the kidney separates the combination of hormone and antihormone, permitting the excretion of gonadotropin and simultaneously retaining antigonadotropins in the circulation.

REFERENCES

1. Zondek, B., and Sulman, F.: The Antigonadotropic Factor, Baltimore, Williams & Wilkins Co., 1942.
2. Rowlands, I. W.: The effect of anti-gonadotropic serum on the reproductive organs of the normal animal, *Proc. Roy. Soc. London*, 121B: 517–532 (Jan.) 1937.
3. Thompson, K. W.: The present status of the antihormone problem, *in* The Chemistry and Physiology of Hormones, Lancaster, Pa., The Science Press Printing Co., 1944, pp. 179–185.
4. Rowlands, I. W., and Spence, A. W.: Production of antigonadotrophic activity in man by injection of extract of pregnant mares' serum, *Brit. M. J.* 2: 947–950 (Nov.) 1939.
5. Jailer, J. W., and Leathem, J. H.: Antigonadotropic substances in man following treatment with pregnant mare serum, *Proc. Soc. Exper. Biol. & Med.* 45: 506–508 (Oct.) 1940.
6. Leathem, J. H., and Rakoff, A. E.: Studies on antihormone specificity with particular reference to gonadotropic therapy in the female, *J. Clin. Endocrinol.* 8: 262–268 (March) 1948.

7. LEATHEM, J. H., and RAKOFF, A. E.: Equine pituitary gonadotrophin and antihormone formation, *Endocrinology* **40**: 454 (June) 1947.
8. HELLER, C. G.; LAUSON, H., and SEVRINGHAUS, E. L.: The immature rat uterus as an assay end-point for gonadotropic substances, *Am. J. Physiol.* **121**: 364–378 (Feb.) 1938.
9. JUNGCK, E. C.; MADDOCK, W. O., and HELLER, C. G.: Gonadotropic hormone, comparison of ultrafiltration and alcohol precipitation methods of recovery from urine, *J. Clin. Endocrinol.* **7**: 1–10 (Jan.) 1947.
10. MEYER, R. K., and WOLFE, H. R.: Gonadotropic inhibitory substance and precipitins in the blood of monkeys receiving gonadotropic hormone preparations, *J. Immunol.* **37**: 91–102 (Aug.) 1939.
11. SPENCE, A. W.; SCOWEN, E. F., and ROWLANDS, I. W.: The absence of antigonadotropic substances in the blood serum of man injected with gonadotropic extracts, *Brit. M. J.* **1**: 66–67 (Jan. 8) 1938.
12. RAKOFF, A. E., and LEATHEM, J. H.: Clinical gonadotropic therapy complicated by antihormone formation, *Fed. Proc.* **5**: 83 (Feb.) 1946.
13. SEGALOFF, A., and PARSON, W.: Hypogonadotropic eunuchoidism: report of case with failure to respond to chorionic gonadotropic hormone due to antihormones, *J. Clin. Endocrinol.* **7**: 130–133 (Feb.) 1947.
14. THOMPSON, K. W., and CUSHING, H.: Inhibition of action of pituitary hormones by animal sera, *Proc. Roy. Soc. London* **121B**: 501–517 (Jan.) 1937 .
15. KUPPERMAN, H. S.; MELLISH, C. H., and MCSHAN, W. H.: Specificity of fowl and mammalian antigonadotropic sera, *Proc. Soc. Exper. Biol. & Med.* **48**: 79–83 (Oct.) 1941.
16. SIMONNET, H., and MICHEL, E.: Recherches expérimentales sur la nature des antihormones, *Compt. rend. Soc. de biol.* **129**: 918–921 (Dec. 10) 1938.
17. OKKELS, H.: The culture of whole organs. III. The problem of antihormones studied on isolated living thyroid glands, *J. Exper. Med.* **66**: 305–316 (Sept.) 1937.
18. SELYE, H.; COLLIP, J. B., and THOMSON, D. L.: Loss of sensitivity to anterior pituitary-like hormone of pregnancy urine, *Proc. Soc. Exper. Biol. & Med.* **31**: 487–488 (Jan.) 1934.
19. SELYE, H.; COLLIP, J. B., and THOMSON, D. L.: Loss of sensitivity to the gonadotropic hormone of the hypophysis, *Proc. Soc. Exper. Biol. & Med.* **31**: 566 (Feb.) 1934.
20. ZONDEK, B., and SULMAN, F.: The antigonadotropic factor. Reversibility of the prolan-antiprolan effect, *Proc. Soc. Exper. Biol. & Med.* **37**: 343–348 (Nov.) 1937.
21. KUPPERMAN, H. S.; MEYER, R. K., and HERTZ, R.: The effect of antigonadotropic sera upon gonadotropic secretion in parabiotic rats, *Endocrinology* **24**: 115–118 (Jan.) 1939.
22. KUPPERMAN, H. S., and MEYER, R. K.: Consideration of the mechanism of neutralization of endogenous gonadotrophic hormone of the rat by antigonadotrophic serum, *Am. J. Physiol.* **145**: 181–185 (Dec.) 1945.
23. BRANDT, R., and GOLDHAMMER, H.: Die Spezifität der gonadotropen Hormone und ihrer Antiseren, *Ztschr. Immunitaetsf.* **88**: 79–90 (May 30) 1936.
24. ZONDEK, B., and SULMAN, F.: Antigonadotrophic factor; origin and preparation, *Proc. Soc. Exper. Biol. & Med.* **36**: 708–712 (June) 1937.

APPENDIX

TABLE A. PATIENT D.B.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 65 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	110	16	4
2	0.9	before therapy	100	29	4
2	—	—	51	11	3
2	0.9	24	53	9	3
4	—	—	109	83	3
4	1.8	39	102	70	3
4	—	—	109	64	3
4	1.8	52	124	32	3
4	—	—	101	77	3
4	1.8	66	107	41	3
4	—	—	93	54	3
4	3.0	81	98	33	3
4	—	—	120	52	3
4	3.0	102	111	21	3
4	6.0	102	115	19	3
4	—	—	90	67	3
4	6.0	151	108	56	3

TABLE B. PATIENT H.B.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 104 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	100	19	3
2	0.9	before therapy	111	31	3
2	—	—	113	37	3
2	0.9	24	103	41	3
2	—	—	138	66	3
2	0.9	51	130	18	3
4	—	—	109	83	3
4	1.8	80	116	36	3
4	—	—	109	64	3
4	1.8	93	129	15	3
4	—	—	101	77	3
4	1.8	105	127	20	3
4	—	—	120	52	3
4	1.8	143	121	27	3
4	3.0	143	122	17	3
4	—	—	90	67	3
4	6.0	192	111	67	3

TABLE C. PATIENT R.C.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 84 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	100	19	3
2	0.9	before therapy	103	17	3
2	—	—	51	11	3
2	0.9	45	72	12	3
4	—	—	109	83	3
4	1.8	60	146	54	3
4	—	—	109	64	3
4	1.8	73	151	20	3
4	—	—	101	77	3
4	1.8	87	41	12	3
4	—	—	120	62	3
4	0.9	137	124	59	3
4	1.8	137	133	48	3
4	—	—	101	50	3
4	3.0	144	154	39	3
4	—	—	111	51	3
4	6.0	209	149	54	3

TABLE D. PATIENT L.D.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 82 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	100	19	3
2	0.9	before therapy	104	28	3
2	—	—	113	37	3
2	0.9	22	107	38	3
2	—	—	134	32	3
2	0.9	36	138	26	3
2	—	—	138	66	3
2	0.9	49	143	12	3
4	—	—	109	83	3
4	1.8	78	143	13	3
4	—	—	109	64	3
4	0.9	91	133	17	2
4	1.8	91	29	12	2
4	—	—	101	77	3
4	0.9	105	130	29	3
4	—	—	120	52	3
4	0.9	141	106	45	3
4	1.8	141	113	26	3
4	—	—	90	67	3
4	1.8	190	97	70	3
4	3.0	190	102	66	3
4	—	—	111	51	3
4	6.0	227	106	53	3

TABLE E. PATIENT C.G.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH daily for 60 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	139	39	4
2	0.9	56	33	12	4
2	—	—	106	39	3
2	0.9	77	41	13	4
2	—	—	98	27	5
2	0.9	95	29	12	5
2	—	—	113	37	3
2	0.9	125	54	17	3
2	—	—	134	32	3
2	0.9	137	27	15	3
4	—	—	109	83	3
4	0.9	180	151	40	2
4	1.8	180	44	16	3
4	—	—	109	64	3
4	1.8	193	36	19	3
4	—	—	93	54	3
4	1.8	222	107	23	3
4	—	—	120	52	3
4	1.8	243	97	15	3
4	3.0	243	28	12	3
4	—	—	101	50	3
4	1.8	264	136	44	3
4	3.0	264	93	20	3
4	—	—	102	64	3
4	3.0	343	110	30	3
4	6.0	343	59	19	3

TABLE F. PATIENT K.H.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH twice daily for 12 days
then 50 units once daily for 54 days

Units sheep F.S.H. per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
2	—	—	110	16	4
2	0.9	before therapy	113	25	4
2	—	—	113	37	3
2	0.9	27	100	25	3
2	—	—	138	66	3
2	0.9	53	41	11	3
2	—	—	51	11	3
2	0.9	67	23	8	3
4	—	—	109	83	3
4	0.9	82	146	15	2
4	1.8	82	37	13	3
4	—	—	109	64	3
4	0.9	95	118	21	3
4	—	—	101	77	3
4	0.9	109	118	31	2
4	—	—	120	62	4
4	1.8	159	114	30	3
4	—	—	90	67	3
4	1.8	194	99	87	2
4	4.5	194	99	47	3
4	—	—	111	51	3
4	6.0	230	127	47	3

TABLE G. PATIENT G.M.: ANTIHORMONE TITERS TO SHEEP FSH
Treatment: 50 units sheep FSH twice daily for 45 days
then 50 units once daily for 11 days

Units sheep FSH per rat	Total cc. plasma per rat	Days after therapy initiated; plasma collected	Assay rats		
			Uterine weight mg.	Ovarian weight mg.	Number of rats
4	—	—	109	83	3
4	1.8	22	95	74	3
4	—	—	101	77	3
4	1.8	45	115	55	3
4	—	—	120	52	3
4	3.0	77	77	18	3
4	—	—	102	64	3
4	6.0	177	85	65	3

TABLE H. CONTROL DATA, TESTING PLASMA FROM NORMAL MEDICAL STUDENTS R.B., L.C. AND E.J. AGAINST SHEEP FSH AND URINARY GONADOTROPINS

Hormone	Plasma: amount and source	Assay rats		
		Uterine weight mg.	Ovarian weight mg.	Number of rats
4 units	—	120	62	4
sheep	6.0 cc.—L.C.	114	52	3
FSH	6.0 cc.—E. J.	121	52	3
12-hour ultrafilter urine concentrate, patient C.G., 7 mo. after stopping therapy	—	89	77	3
	6.0 cc.—R.B.	107	86	3
12-hour ultrafilter urine concentrate, patient K.H., 4 mo. after stopping therapy	—	125	23	3
	6.0 cc.—L.C.	132	14	3
12-hour ultrafilter urine concentrate, patient L.D., 3 mo. after stopping therapy	—	107	69	3
	6.0 cc.—E. J.	137	71	3
12-hour ultrafilter urine concentrate, normal medical student, E.J.	—	105	44	3
	6.0 cc.—E. J.	112	35	3

THE PREGNANDIOL PRECIPITATION TEST. FURTHER OBSERVATIONS ON CLINICAL APPLICATIONS AND TECHNIC*

HAROLD C. MACK, M.D., ARTHUR E. PARKS, B.S. AND MARIAN McDONALD, B.A.

From the Endocrine Laboratory and the Department of Obstetrics and Gynecology, Harper Hospital, Detroit, Michigan

THE value of the pregnandiol precipitation test as a rapid laboratory aid in the diagnosis of pregnancy and as a means for predicting the probable outcome of threatened abortion has been indicated in previous reports (1, 2, 3, 4). Added experience with the method has since led to certain modifications of technic. An appraisal of its efficiency based on 737 pregnancy tests and a study of 149 patients with threatened abortion is now presented. Further possible applications of the method in investigation of normal and abnormal ovarian cycles are suggested.

Briefly stated, the pregnandiol test developed in this laboratory depends upon the precipitation of unpurified pregnandiol from the urine of women during the postovulatory phases of normal menstrual cycles and during amenorrhea of normal pregnancy. The presence of pregnandiol during the ovarian cycle is indicative of antecedent ovulation; its presence during amenorrhea of normal pregnancy indicates presumably normal corpus luteum and placental function. The physiologic basis for these assumptions has been well established by numerous investigations using other technics, principally the more time-consuming quantitative methods. Our studies, using the greatly simplified qualitative precipitation test, closely parallel the findings of other investigators.

Recent applications of the precipitation method in studies of normal and abnormal menstrual cycles, correlating the excretion of pregnandiol to fluctuations of basal body temperature, have shown a close relationship of pregnandiol excretion to the postovulatory temperature rise. In cycles with typical biphasic temperature patterns, we have consistently observed pregnandiol excretion following presumptive ovulation.

The amounts of pregnandiol precipitate varied considerably. Pregnandiol excretion occurred most commonly between the second and fifth days following ovulation and disappeared usually one to four days before the onset of the menstrual flow and the fall of morning temperature.

Received for publication July 23, 1948.

* Aided by a grant from the Stewart Hamilton Memorial Research Fund of Harper Hospital.

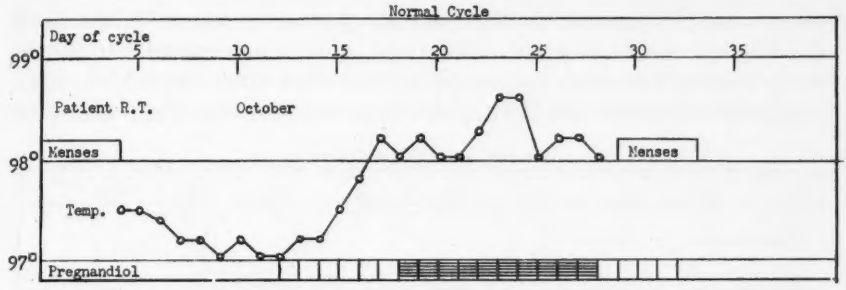


FIG. 1. Open squares indicate days when pregnandiol precipitation test was negative. Shaded squares indicate days when pregnandiol precipitation test was positive.

Figure 1 graphically illustrates a typical relationship between basal temperature and pregnandiol excretion in a normal subject, Mrs. R. T., a 24-year-old white married female who became pregnant three months later. The time of appearance of the precipitate following the postovulatory temperature rise and its absence on the day preceding the menses is typical of our findings in other normal, ovulatory cycles.

Figure 2 (Mrs. A. D., para 1, gravida 2) illustrates an instance in which the relationship of pregnandiol excretion to basal body temperature was observed during the cycle preceding the onset of a planned pregnancy. Continued excretion of pregnandiol and the sustained temperature level following ovulation are typical of normal pregnancy.

Figures 1 and 2 illustrate normal cycles of regular interval in which the ovulatory temperature rise and postovulatory pregnandiol excretion occur in typical sequence.

Figure 3 (M. D., para 0, gravida 0) illustrates an example of delayed ovulation and late pregnandiol excretion in a subject whose menstrual

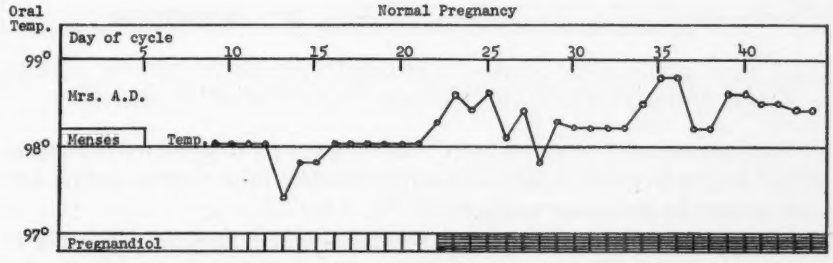


FIG. 2. Open squares indicate days when pregnandiol precipitation test was negative. Shaded squares indicate days when pregnandiol precipitation test was positive.

cycles usually conformed to the "normal" pattern as shown in the April and May curves. During the June cycle, ovulation occurred late in the fourth week (26th day) instead of at the usual time (14th-16th day). Pregnandiol excretion was first observed on the 28th day. Late ovulation

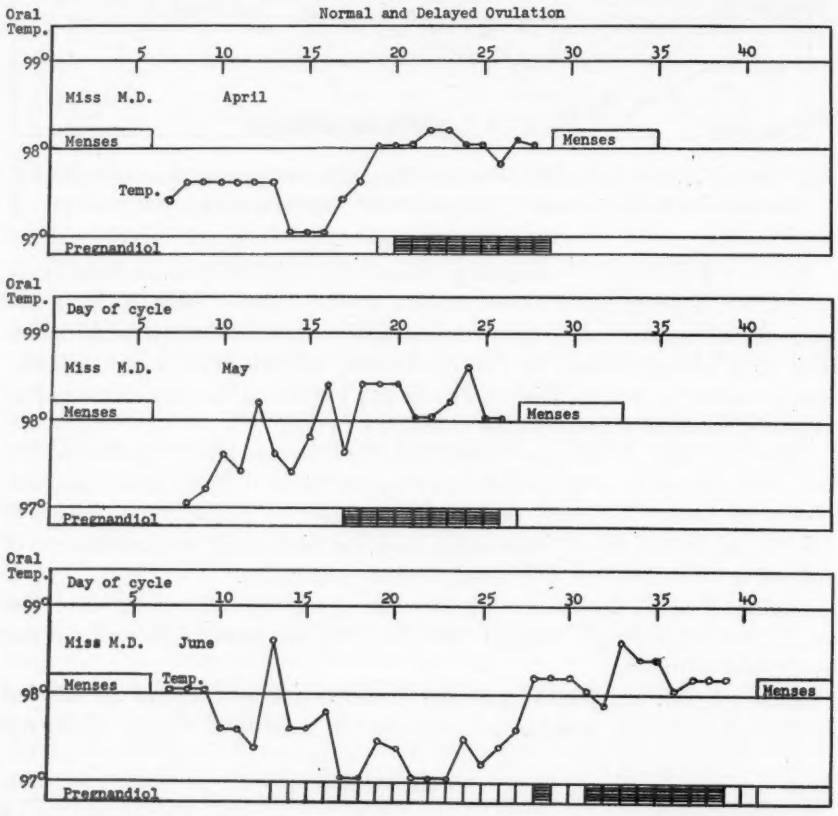


FIG. 3. Open squares indicate days when pregnandiol precipitation test was negative. Shaded squares indicate days when pregnandiol precipitation test was positive.

and late pregnandiol excretion were thus followed by delayed menstruation on the 42nd day. Such occurrences explain certain false positive pregnancy tests as will be discussed below.

Figure 4 illustrates data in a patient (Mrs. W. R. T., para 1, gravida 1) under study because of sterility of five years' duration. In March she exhibited a 29-day cycle with complete absence of pregnandiol excretion; in

April and May the cycles were 37 and 41 days respectively with normal pregnandiol excretion.

Our studies of pregnandiol excretion in instances of sterility have shown numerous cycles in which absence of the typical biphasic temperature shift

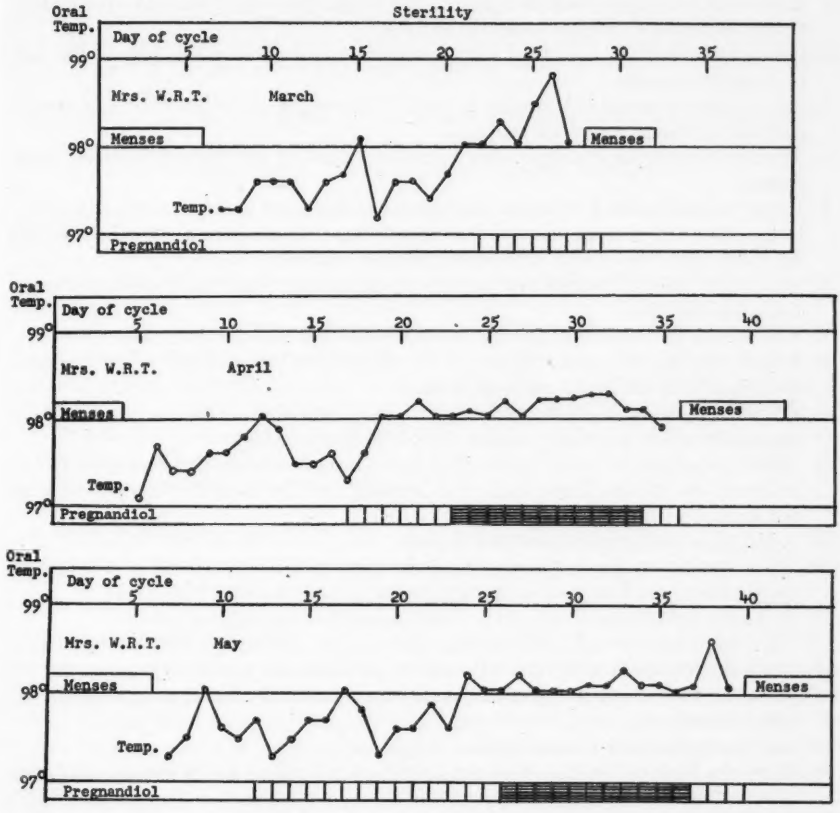


FIG. 4. Open squares indicate days when pregnandiol precipitation test was negative. Shaded squares indicate days when pregnandiol precipitation test was positive.

and lack of pregnandiol excretion appear to offer some explanation for infertility on the basis of failure of ovulation. The estimation of pregnandiol in the study of patients with disturbances of menstruation or infertility may thus offer information of considerable diagnostic importance. Detailed analysis of these studies will be presented in a subsequent report.

TECHNIC

Experience gained in performing more than 6000 determinations has led to certain valuable modifications of the procedure:

1. Obtain an 8-hour overnight urine specimen.
2. Place the *entire* specimen in an Erlenmeyer flask of convenient size.
3. Add 50 ml. of toluene (reagent quality), 10 volumes per cent of concentrated hydrochloric acid, and a few glass beads to the flask.
4. Connect the flask to a vertical Liebig condenser and reflux vigorously on the hot plate for 15 minutes.
5. Remove the flask and cool rapidly to room temperature under the water tap. Avoid agitation of the sample while cooling.
6. Transfer the sample to a separatory funnel and draw off and discard the lower layer (urine).
7. If only a small amount of emulsion is present at this point it can be disregarded. If a large amount is present, transfer the urine-toluene emulsion to a 100 ml. tube and centrifuge for 5 minutes at 1500 revolutions per minute.
8. Add 15 ml. of 0.1 N sodium hydroxide to the separatory funnel and wash by swirling the sample gently.
9. The sodium hydroxide solution will settle to the bottom and is discarded.
10. Repeat step #8 with an additional 15 ml. of sodium hydroxide and follow the same procedure with two 15 ml. portions of water.
11. After the last washing with water make a very careful separation so that only the toluene layer is transferred to a *dry* 125 ml. Erlenmeyer flask.
12. Allow the sample to stand undisturbed for at least 5 minutes and then pour it into a second dry 125 ml. Erlenmeyer flask making sure that any droplets of water are left behind.
13. Add 2 glass beads and boil on the hot plate.
14. When the vapors reach the mouth of the flask, add slowly 10 ml. of 2% sodium hydroxide in absolute methanol while the flask remains on the hot plate.
15. Boil until the toluene is reduced to about one-half of the original volume.
16. Filter while hot through a fritted glass filter (Pyrex, medium porosity) using suction.
17. Place the filtrate in a dry 125 ml. Erlenmeyer flask and take to dryness on the hot plate. To avoid overheating of the residue use a gentle air stream to remove the last traces of toluene.
18. Add 5 ml. of acetone to the residue.
19. Place the flask on the hot plate and add 25 ml. of boiling 0.1 N sodium hydroxide, slowly, 3 to 4 ml. at a time. After the addition of the first 3 to 4 ml. of the sodium hydroxide solution to the 5 ml. of acetone, reduce the volume to approximately 3 ml. before addition of the next portion. The remainder of the sodium hydroxide may be added more rapidly—shaking the flask and bringing to a boil between the addition of each portion.
20. Remove the sample from the hot plate and observe in a good light. In strongly positive samples a macroscopic, white, flaky precipitate will be seen in the solution and particularly on the side of the flask above the level of the liquid. If a precipitate is present and plainly seen at this stage, record as a four-plus reaction and carry the procedure no further. In case of no precipitate or a questionable result continue with step #21.
21. Place the flask in the refrigerator or an ice bath until cold.

22. Transfer 1 ml. of the sample to a Kahn tube and allow it to come to room temperature.
23. Read in the following manner using a Kahn viewing device:[1]
    (a) A "two-plus" reaction shows a very fine precipitate which requires careful scrutiny of the tube to be visible.
    (b) A "three-plus" reaction is still a fairly fine precipitate but is readily evident upon looking at the tube in the viewing box.
    (c) A "four-plus" reaction is reserved for those reactions which show an agglutination of the precipitate into large particles, or for those which were positive in step #20.
24. Add one drop of concentrated hydrochloric acid to tubes which still show a negative or questionably positive reaction. Some of these now show a precipitate which floats on the surface of the liquid. Record this as a "one-plus" reaction.

Reagents

1. 0.1 *N sodium hydroxide* (approximately):—Prepare a stock solution of 2 N sodium hydroxide by making 160 grams of sodium hydroxide pellets C.P. up to 2 liters with distilled water. For approximately 0.1 N sodium hydroxide, make 100 ml. of the stock solution up to 2 liters with distilled water. These solutions are not critical. No further standardization is necessary.
2. 2% *sodium hydroxide in absolute methanol*:—Dissolve approximately 2 grams of sodium hydroxide pellets C.P. in 100 ml. of absolute methanol (synthetic or C.P.). Prepare fresh daily and filter before use.
3. Hydrochloric acid C.P.
4. Toluene (purified or reagent quality).
5. Acetone C.P.
6. Methyl alcohol, absolute C.P.

MODIFICATIONS

Eight-hour specimen (step #1).

We originally followed Guterman's example (5, 6) in using 100 ml. of a first morning specimen of urine for assay. It was soon evident, however, that a more constant aliquot of the 24-hour urine excretion was necessary, since overnight samples varied from 50 to 1500 ml. Since, also, the daily pregnandiol output during the luteal phase of the cycle and early pregnancy has been shown to vary from 1.5 to 10 mg. (7), and since our technic accurately detects 0.5 mg. of pregnandiol (1), consistently positive results in women excreting a minimum of 1.5 mg. per day could not be expected unless one-third of the daily output was analyzed. Accordingly, we now insist upon 8-hour samples. The consistent use of 8-hour specimens makes it possible to compare the amounts excreted by different women or by the same woman from day to day. Somerville, Gough, and Marrian (8) also found 100 ml. samples of urine inadequate for pregnandiol assay and suggested using one-fifth of a 24-hour specimen.

---

[1] Sell, Floyd: "Viewing Device for the Final Stage of the Kahn Test." Presented before the Illuminating Engineering Society, Great Lakes Regional Conference, June 5, 1943.

Care of fritted glass filter (step #16).

The same fritted glass filter has been used continuously for approximately 6000 determinations of pregnandiol performed in this laboratory in the past two years. After each filtration the surface precipitate is washed off under the hot water tap and about 30 ml. of hot water is drawn through the filter by suction. This is followed by a few ml. of 95 per cent ethyl alcohol and lastly 5 ml. of acetone. By following this procedure the filter is left clean and dry for the next filtration. Once a week concentrated sulfuric acid is used to remove any accumulated organic material.

Addition of sodium hydroxide (step #19).

We wish to re-emphasize the importance of slow initial addition of the sodium hydroxide solution in step #19. After addition of the first 3 to 4 ml. of sodium hydroxide the volume is reduced by boiling to a total of approximately 3 ml. before addition of the next portion. The remainder of the solution may then be added more rapidly.

Delayed precipitation.

We have consistently given the impression that the pregnandiol precipitation test can be completed in 1 hour or less. However, this is only true of those samples giving an immediate positive result in step #20 or #24. It has been observed that occasionally a reaction originally interpreted as negative will have become positive upon re-inspection the following morning. Inasmuch as routine samples need not be rushed to completion we place the flasks from step #20 in the refrigerator as they are completed. Before leaving the laboratory at night one ml. of each sample is transferred to a Kahn tube and allowed to remain at room temperature until morning. The results are then read in the Kahn viewing device and recorded as negative, questionable, two-plus, three-plus, or four-plus.

Use of hydrochloric acid in precipitation (step #24).

One drop of concentrated hydrochloric acid is added to tubes showing negative, questionable, or weak positive reactions and after agitating, the tubes are again scrutinized. Some reactions which were read as negative or questionable will now show a precipitate floating on the top of the liquid. These are recorded as one-plus. By the use of acid it has been possible to make the interpretation of the reaction more objective and to reduce the number of false negative results without increasing the number of false positives.

RESULTS

The pregnandiol precipitation test has been employed in our laboratory since May, 1946, in conjunction with the Friedman test on all urine samples submitted for the diagnosis of pregnancy. From May, 1946 to April, 1948, 737 tests have been performed. Following the reports of Guterman (5, 9) concerning the prognostic significance of pregnandiol excretion levels in cases of threatened abortion, we have applied our method to a similar study of 149 patients.

Normal Pregnancy (Table 1). Urine samples from 381 women subsequently found to have been pregnant were submitted for diagnosis. Of these, 361 gave correct positive pregnandiol readings (94.8%); 20 gave incorrect negative readings (5.2%). The Friedman test performed simultane-

TABLE 1

Normal pregnancy

Duration of amenorrhea (from expected menstrual period to time of test)	Pregnandiol precipitation test		Friedman test	
	False negative	Correct positive	False negative	Correct positive
Less than 1 week	1	18	3	16
1 to 2 weeks	4	60	1	57
2 to 3 weeks	3	46	1	44
3 to 4 weeks	3	46	3	41
Over 4 weeks	6	132	3	117
Unknown	3	59	2	58
	20 (5.2%)	361 (94.8%)	13 (3.8%)	333 (96.2%)
	Total 381		Total 346	

TABLE 2

Nonpregnant states

Condition	Pregnandiol precipitation test		Friedman test	
	False positive	Correct negative	False positive	Correct negative
Delayed menses	18	59	1	70
Secondary amenorrhea	1	11	0	12
Functional bleeding	0	14	1	10
Menopause	2	13	0	13
Postabortion	0	18	0	8
Lactation	1	5	0	6
Pelvic inflammatory disease	1	25	0	3
Fibroids	3	20	0	12
Ovarian cysts and tumors	4	8	0	7
Pseudocyesis	0	3	0	0
Insufficient data	10	140	3	136
	40 (11.2%)	316 (88.8%)	5 (1.8%)	277 (98.2%)
	Total 356		Total 282	

ously (in instances where sufficient urine was available) gave correct positive reactions in 333 of 346 specimens (96.2%); false negative tests were obtained in 13 (3.8%). The accuracy of these tests was determined by

subsequent reports from physicians who submitted the samples for diagnosis.

Nonpregnant states (Table 2). Urine samples from 356 patients subsequently determined not to have been pregnant at the time of testing gave correct negative pregnandiol reactions in 316 (88.8%); false positive tests were reported in 40 (11.2%). The Friedman test gave correct negative reactions in 277 of 282 tests (98.2%). There were 5 false positive Friedman reactions (1.8%).

False positive pregnandiol tests (Table 3). The case histories of patients with false positive pregnandiol reactions were subjected to further scrutiny after the clinical results became known. The 18 patients classified as "delayed menses" had gone more than seven days beyond the date of the expected period when the tests were made; 13 of these patients always menstruated at irregular intervals. The possibility of late ovulation and consequently delayed menstruation in such instances has been illustrated

TABLE 3

FALSE POSITIVE PREGNANDIOL TESTS

No amenorrhea	18
Delayed menses	18
Corpus luteum cyst	2
Lactating	1
Secondary amenorrhea	1
Total	40

in Figures 3 and 4. In 18 of the 40 cases, specimens were submitted during the luteal phases of normal cycles because of pelvic findings (tumor, adnexal enlargement) suggesting to the physician the possibility of pregnancy, or because of abnormal flow during the preceding cycle. In two instances positive tests were obtained in patients operated upon for corpus luteum cysts.

It is evident from the foregoing and from other reports in the literature (6, 10, 11) that the positive pregnandiol test should be interpreted with caution since it gives presumptive evidence of pregnancy *only when the urine is obtained during periods of amenorrhea in women with otherwise normal menstrual cycles.* When these criteria are followed, the accuracy of the pregnandiol test in nonpregnant states compares favorably to that in normal pregnancy. In cycles regularly surpassing the usual 28-day length (Figs. 3 and 4), late pregnandiol excretion as a consequence of late ovulation may be expected. These fundamental facts cannot be over-emphasized since lack of their understanding has led to false interpretations of the test. When cycles are consistently irregular, the Friedman test should be performed simultaneously.

TABLE 4

THREATENED ABORTION

Retention of fetus—68 patients

No. of weeks gestation	5–8	9–12	13–16	17–20	Unknown	Total
Pregnandiol test positive	23	28	8	7	0	66
Pregnandiol test negative	2	0	0	0	0	2

Abortion—81 patients

No. of weeks gestation	5–8	9–12	13–16	17–20	Unknown	Total
Pregnandiol test positive	8	11	7	3	1	30
Pregnandiol test negative	14	30	2	4	1	51

Threatened abortion. Table 4 shows the results of a study in 149 patients with symptoms of threatened abortion (cramps, bleeding) during early pregnancy. In 51 cases, negative pregnandiol tests preceded the occurrence of spontaneous abortion. In 10 instances, positive pregnandiol tests became negative during the period of observation in cases which similarly terminated in fetal loss. In 66 cases, pregnandiol tests remained positive during the period of symptoms in cases in which the fetus was retained. Incorrect prediction was made in 30 cases in which the tests remained positive despite eventual abortion. Nineteen of the 30 cases were in the first trimester of gestation when abortion took place. The possibility that positive tests in some instances were associated with nonendocrine causes of abortion must also be considered. However, while the pregnandiol precipitation test evidently provides rapid screening, it may be necessary to resort to quantitative methods for the detection of decreasing pregnandiol levels in instances where persistent positive reactions are obtained despite continuation of symptoms.

The qualitative pregnandiol precipitation test has its greatest value during threatened abortion prior to the fifth month of gestation. Since increasing placental function adds greatly to the output of pregnandiol after the fourth month it may also be necessary to rely upon quantitative methods to determine diminished progesterone metabolism in later stages. Of the 53 patients giving negative results with this test, 51 or 96.2 per cent aborted. From these data it seems evident that in pregnancy negative pregnandiol tests are almost pathognomonic of subsequent abortion.

SUMMARY AND CONCLUSIONS

The pregnandiol precipitation test, performed in 737 urine specimens submitted for the rapid diagnosis of pregnancy showed an over-all efficiency of 91.9 per cent. In cases of normal pregnancy the accuracy was 94.8 per cent, whereas in nonpregnant states it was 88.8 per cent. False positive tests reported in women subsequently found not to have been

pregnant could be explained in part by delayed pregnandiol excretion consequent to late ovulation in irregular cycles; or by the urine specimen having been procured during the luteal phases of normal cycles. When these cases are eliminated and the test is restricted to instances of genuine amenorrhea, the accuracy in nonpregnant states is 97.2 per cent and it thus compares closely to that obtained during normal pregnancy. *Pregnandiol excretion offers presumptive evidence of pregnancy only during amenorrhea of previously normally menstruating women.* When cycles are consistently prolonged or irregular, the Friedman test should be performed in conjunction. The Friedman test showed an accuracy of 96.2 per cent in normal pregnancy and 98.2 per cent in nonpregnant states.

The prognostic value of the pregnandiol precipitation test in threatened abortion has been investigated in 149 cases. Of 53 patients giving negative test results, 51 (96.2 per cent) aborted. From this it seems evident that negative pregnandiol tests during pregnancy are practically pathognomonic of subsequent abortion.

Preliminary investigations of pregnandiol excretion during the postovulatory phases of menstrual cycles, in conjunction with basal temperature determinations, indicate further usefulness of the test in the study of problems of infertility.

REFERENCES

1. PARKS, A. E., and MACK, H. C.: A rapid qualitative test for pregnandiol, *Harper Hosp. Bull.* **4**: 165–168, 1946.
2. MACK, H. C., and PARKS, A. E.: Clinical application of pregnandiol determinations in threatened abortion, *Harper Hosp. Bull.* **5**: 67–76, 1947.
3. MACK, H. C., and PARKS, A. E.: The pregnandiol precipitation test. Clinical application of a rapid method for the diagnosis of pregnancy, *J. Clin. Endocrinol.* **7**: 351–363 (May) 1947.
4. MACK, H. C.; PARKS, A. E., and McDONALD, M.: Further observations on the pregnandiol precipitation test, *Harper Hosp. Bull.* **6**: 33–41, 1948.
5. GUTERMAN, H. S.: A human pregnancy test based upon a color reaction of pregnandiol in the urine, *J. Clin. Endocrinol.* **4**: 262–267 (June) 1944.
6. GUTERMAN, H. S.: Further observation on the value of the pregnandiol test for pregnancy, *J. Clin. Endocrinol.* **5**: 407–411 (Dec.) 1945.
7. BROWN, J. S. L.; HENRY, J. S., and VENNING, E. H.: The significance of endocrine assays in threatened and habitual abortion, *Am. J. Obst. & Gynec.* **38**: 927–955, 1939.
8. SOMERVILLE, I. F.; GOUGH, N., and MARRIAN, G. F.: Estimation of pregnandiol in urine, *Lancet* **2**: 701, 1947.
9. GUTERMAN, H. S.: Prediction of fate of threatened abortion by pregnandiol, *J.A.M.A.* **131**: 378–382 (June 1) 1946.
10. McCORMACK, GRACE: A comparison of the color chemical test with the Friedman modification of the Aschheim-Zondek test, *Am. J. Obst. & Gynec.* **51**: 722–725, 1946.
11. MORROW, A. G., and BENUA, R. S.: An evaluation of the Guterman pregnancy test, *Am. J. Obst. & Gynec.* **51**: 685–691, 1946.

CARBOHYDRATE METABOLISM IN THE COMBINATION OF DIABETES MELLITUS AND ADDISON'S DISEASE, AS ILLUSTRATED BY A CASE

JOSEPH H. CRAMPTON, M.D., SIDNEY T. SCUDDER, M.D., AND CLARENCE D. DAVIS, M.D.

From the Department of Medicine, University of Washington School of Medicine and the Virginia Mason Hospital, Seattle, Washington

SIMULTANEOUS occurrence of Addison's disease and diabetes mellitus is rarely observed. It is surprising that this is only the nineteenth report of a proven occurrence of these two diseases in the same patient, when 28.4 per cent of patients with diabetes mellitus also have active tuberculosis (1). We have combined those cases summarized by Thorn and Clinton in 1943 (2) with the subsequent case reports in Tables 1, 2, and 3.

In both Addison's disease and diabetes mellitus there are marked aberrations in carbohydrate metabolism. A better understanding of these diversions in the individual disease and realization of the characteristic pattern of their summation should lead to premortem diagnosis of adrenocortical insufficiency in a greater number of diabetic patients. To clarify this pattern, a synthesis is presented to illustrate the various abnormalities in carbohydrate and related metabolism. Special emphasis is placed on the biochemical and clinical modifications appearing in each disease.

TABLE 1. PATIENTS WITH SIMULTANEOUS ONSET OF DIABETES MELLITUS AND ADDISON'S DISEASE

Author		Patient's Age yrs.	Patient's Sex	Duration of disease yrs.	Pathologic findings in adrenal gland	Insulin requirements
Arnett	(3) 1927	39	F	3	Atrophy	20–50 units daily
Levy-Simpson	(4) 1932	16	M	1	Atrophy	10 units resulted in hypoglycemia
Gowen	(5) 1932	54	F	1–5	Atrophy	5 units resulted in hypoglycemia
Koepf & Bowen (unpublished)	(2)	20	M	2	Atrophy	diabetes controlled by diet
Nix	(6) 1943	39	M	1½	Atrophy	not controlled

Received for publication July 12, 1948.

TABLE 2. PATIENTS WITH DIABETES MELLITUS WHO SUBSEQUENTLY DEVELOPED ADDISON'S DISEASE

Author*	Patient's		Duration of diabetes at time of onset of Addison's disease yrs.	Apparent etiology of Addison's disease	Duration of Addison's disease
	Age yrs.	Sex			
Unverricht (7) 1926	32	M	6	Tuberculosis	2–4 months
Rowntree & Snell (8) 1931	25	M	1	Atrophy	15 months
Rowntree & Snell (8) 1931	—	M	6	Atrophy	18 months
Rogoff (9) 1936	25	M	4	Denervation of adrenals	12 months
Bloomfield (10) 1939	30	M	1	? Atrophy	Patient alive
Bowen, *et al.* (11) 1942	76	F	10	Tuberculosis	12 months
McCullagh (12) 1942	—	M	7	? Atrophy	Patient alive
Bickel (13) 1945	29	M	6	?	Patient alive
Devitt & Murphy (14) 1947	28	F	3½	Atrophy	3 months
Adler (15) 1947	20	F	2	? Atrophy	Still living

* Since this article was written, we have the following references to add to this category:

Allan (16) 1930—a review only.

Brookfield and Corbett (17) 1934—diagnosis of Addison's disease questionable.

Heim (18) 1940.

Sprague *et al.* (19) 1947—three cases, reported in a brief abstract.

TABLE 3. THOSE PATIENTS IN WHOM ADDISON'S DISEASE PRECEDED DIABETES MELLITUS

Author*	Patient's		Duration of Addison's disease prior to diabetes mellitus years	Etiology of Addison's disease	Duration of combined diseases
	Age yrs.	Sex			
Rhind & Wilson (20) 1941	32	F	2	Atrophy	2 mo.—died
Thorn & Clinton (2) 1943	23	M	4½	Unknown	Alive
Soffer & Sorkin (21) 1945	42	M	2	Unknown	Alive

* Since this article was written, we have the following references to add to this category:

Lowrie *et al.* (22) 1948.

Knowlton and Kritzler (23) 1949.

Carbohydrate metabolism in diabetes mellitus. Diabetes mellitus, fundamentally, results from a deficiency of the hormone insulin. Cori and Cori (24) have recently centered attention upon the enzyme hexokinase and its probable role in facilitating the reaction: glucose plus adenosine triphosphate yields glucose-6-phosphate plus adenosine diphosphate. This reaction represents the first step in the body's utilization of glucose. It is inhibited by the diabetogenic hormone of the anterior pituitary. Insulin acts as an antagonist to the diabetogenic hormone, and has no effect in its absence (25). Therefore, the apparent and perhaps sole effect of insulin is to augment this reaction.

A deficiency of insulin leads to the following secondary biochemical alterations in carbohydrate metabolism: a) increased glycogenolysis, b) decreased rate of utilization of glucose in muscle (26) (the rate approaches normal only at high concentrations of glucose (27)), and c) increased gluconeogenesis (28). Clinically, these are manifested by: a) hyperglycemia, b) glycosuria, and c) glucose tolerance curves with a rapid rise and prolonged elevation in the blood sugar level.

Controlled diabetes mellitus has no specific effect upon protein metabolism, but protein catabolism is increased during uncontrolled periods by the impaired capacity of the organism to utilize glucose and by the resulting hyperglycemia and glycosuria. This chain of events leads to accelerated gluconeogenesis from protein, demonstrated by a negative nitrogen balance and increased urinary nitrogen excretion (29).

The frequency of lipemia in diabetes mellitus indicates alterations in fat metabolism. The exact mechanism involved is unknown, but it probably results from increased mobilization of body lipids secondary to conversion of lipids to glucose. Also, diminished lipogenesis from carbohydrate has been demonstrated (30). Clinically and pathologically these changes are manifested by: a) ketonemia, b) ketonuria, c) excessive deposition of fat in the liver, and d) lipemia.

Carbohydrate metabolism in Addison's disease. In Addison's disease the principal defect in carbohydrate metabolism is the inability to derive glucose from endogenous and exogenous sources. Adrenal cortical hormones have been shown to have an inhibitory effect upon the hexokinase reaction similar to that of the diabetogenic hormone of the anterior pituitary (24). This defect is represented by: a) diminished conversion of glucose to glycogen in both liver and muscle (31, 32); b) no decrease in the utilization of glucose in muscle (33, 34); c) decreased gluconeogenesis from protein (35) and fat (36); d) decreased rate of conversion of glycogen to glucose; and e) reduced absorption of glucose from the gastro-intestinal tract (37). The last phenomenon is probably the result of changes in physical absorption of

materials from the gut, secondary to the lack of salt-active hormones of the adrenal cortex.

Clinically, these alterations are characterized by: a) extreme insulin sensitivity, b) fasting hypoglycemia, c) flat oral glucose tolerance curve, d) an intravenous glucose tolerance curve which shows a normal initial rise and then a fall to moderate or severe hypoglycemic levels, e) hypoglycemic reactions which tend to be more severe at higher levels of the blood sugar than in the normal individual (37), and f) a failure of epinephrine to produce a normal rise in the blood sugar (37).

Protein metabolism is significantly changed in Addison's disease. In untreated chronic adrenocortical insufficiency, conversion of protein into glucose diminishes (35). Conversely, it is noted that after administration of certain fractions (Kendall compounds A, B, and E) of adrenocortical extract, excretion of nitrogen in the urine increases, denoting accelerated protein catabolism (38).

In Addison's disease impairment of fat metabolism is shown by: a) diminished ability to utilize the intermediate products of fatty acid metabolism (36); b) lessened formation of ketone bodies after injection of phlorhizin (39); and c) inability to deposit fat in the liver (40). It should be noted that the tendency toward acidosis often found in Addison's disease may increase when diabetes mellitus supervenes. This is probably the result of two factors: diminished ability to utilize the intermediate products of fatty acid metabolism, and excessive excretion of sodium by the kidneys due to lack of salt-active adrenal hormones.

The case to be presented illustrates the interaction of the many and diverse physiochemical processes described above.

CASE HISTORY

First Admission: On July 8, 1942, L. R., a 56-year-old woman, was referred to the Mason Clinic for control of diabetes mellitus of sixteen years' duration, for which she had been taking crystalline insulin (C), 20 units, before each meal. The past history disclosed that in 1920 a goiter was removed because of symptoms of toxicity. Her general state of health had remained good.

Physical examination revealed a blood pressure of 108 systolic and 68 diastolic, a dry and sallow skin with a vague bluish tint; there were no other significant physical findings. The urine showed a 2-plus sugar and 1-plus albumin. The blood hemoglobin was 14 Gm.; erythrocyte count, 5,000,000; and leukocyte count, 7,300, with a normal differential count. The blood Kahn and Kolmer tests were negative. Blood sugar levels ranged from 108 to 385 mg. per cent. A roentgenogram of the chest demonstrated calcified mediastinal lymph nodes.

The patient was discharged on a diet of 175 Gm. of carbohydrate, 80 Gm. of protein and 100 Gm. of fat; and was to take 20 units of protamine zinc insulin (PZI) and 10 units of crystalline insulin (C-10) before breakfast.

Interval: Ten months later she returned complaining of mild nausea. This continued

intermittently over a period of seventeen months despite minor adjustments in insulin dosage. Three years and four months after discharge she contracted mumps. She was hospitalized elsewhere and given 20 units of globin insulin daily. Later, afternoon hypoglycemic reactions were noted, even though the patient decreased the globin insulin to 12 units daily. At the same time, she experienced progressive weakness, anorexia, weight loss (8 pounds), increasing malaise, intermittent diarrhea, and deepening facial pigmentation. However, she continued to work until vomiting started two days before readmission.

Second Admission: July 28, 1946 (four years after first admission). The patient appeared chronically ill and weak. A dirty brownish pigmentation of the face and shoulders without discoloration of the mucous membrane of the mouth or the breast areolae was noted. The blood pressure was 110 systolic and 70 diastolic, and the pulse rate was 80.

Urinalysis showed a moderate number of leukocytes. The complete blood count was normal. The blood chloride (expressed as sodium chloride) was 263 mg. per cent; the sugar, 504 mg. per cent; the cholesterol, 400 mg. per cent; and the carbon dioxide combining power, 60 volumes per cent. The bromsulphalein liver function test (5 mg. per Kg.) showed less than 5 per cent retention in forty-five minutes. A roentgenogram of the abdomen revealed a small calcification just to the left of the transverse process of the second lumbar vertebra. X-ray studies of the stomach, duodenum, and gall bladder showed these organs to be normal. Basal metabolic rates were minus 21 and minus 30 per cent. An electrocardiogram demonstrated a rate of 86, a PR interval of 0.19, a low R-1, inverted T-2 and T-3, isoelectric T-4 and a flat T-1. The voltage was at the lower limits of normal.

Extreme weakness, malaise, and intolerance to food with nausea and vomiting continued. She was too weak to rise from the supine position. Many mornings she was found in a comatose state from which she responded only after the administration of glucose in physiologic saline. The systolic blood pressure ranged from 88 to 54. The blood sugar during one such episode was 91 mg. per cent.

At first the patient was maintained on intravenous fluids. Then, after a short interval of aqueous adrenocortical extract administration, she was given 4 mg. daily of desoxycorticosterone acetate (DCA) in oil, intramuscularly, supplemented by 3 Gm. of sodium chloride orally. She remained somewhat nauseated; therefore the DCA was increased to 7 mg. On this regimen she had no nausea or vomiting, and her appetite was excellent. She became ambulatory, and was bright and alert, in marked contrast to her previous lethargy.

As soon as DCA therapy was begun, she started to have severe hypoglycemic reactions before and after breakfast. Sugar determinations on blood obtained before breakfast were near 50 mg. per cent. Her diabetes mellitus had been controlled on PZI, 12 to 16 units daily, but hypoglycemic reactions which followed treatment with DCA forced a reduction to a maintenance dose of PZI, 8 units daily. After one week on this regimen the blood pressure rose to 135 systolic and 80 diastolic, and the blood chlorides to 462 mg. per cent. Reversion toward normal was noted in the electrocardiogram.

In an effort to establish the diagnosis more conclusively, DCA was withdrawn and placebo hypodermic injections were substituted. Salt was withdrawn from the diet and none given additionally. At the end of sixty hours the Robinson-Power-Kepler water diuresis test was performed. The day excretion of urine equalled the night excretion, and the final equated figure was 23. A sodium determination on serum taken during this interval was 310 mg. per cent (the normal blood sodium in our laboratory is 330–340 mg. per cent), and the blood chlorides were 478 mg. per cent. During the withdrawal

of adrenal hormone therapy, there was progressive deterioration to her former state. Again this was relieved almost immediately by intensive treatment with intravenous saline, DCA, and adrenocortical extract. She was well enough to be discharged seventy-two hours after the reinstitution of therapy. Her discharge diet was carbohydrate 165 Gm., protein 80 Gm., and fat 90 Gm. She was instructed to take daily, 7 mg. of DCA normal dietary salt, 3 Gm. of supplementary salt, and PZI, 8 units.

Interval: One month later, because of progressive weight increase and edema of the ankles, the DCA was reduced to 3 mg. daily and the salt was discontinued. Apathy, nausea, vomiting, malaise and weakness soon reappeared and progressed, even though the dosage of DCA was increased to 6 mg. daily.

Third Admission: November 26, 1946 (four months after second admission). She was unable to give a coherent story. There was an increase in skin pigmentation and, for the first time, a dusky hue of the oral mucous membrane was observed. Her blood pressure was 155 systolic and 100 diastolic. There was no evidence of heart failure or edema.

The blood hemoglobin was 12 Gm.; erythrocyte count, 3,600,000; and the leukocyte count, 5,750. Urinalysis showed a few leukocytes and a slight amount of albumin. The blood chloride was 429 mg. per cent; nonprotein nitrogen, 25 mg. per cent; serum sodium 315 mg. per cent; and serum protein, 5.01 Gm. per cent, with an albumin-globulin ratio of 2.01. The DCA was increased from 6 to 12 mg. In addition, 20 to 30 cc. of aqueous adrenocortical extract and 18 Gm. of sodium chloride were given intravenously daily. During the last four days of life this was supplemented each day with 4 to 6 cc. of lipoadrenal extract, given intramuscularly. Throughout she received PZI. 8 units each morning before breakfast.

The blood pressure remained between 160 systolic and 80 diastolic, and 170 systolic and 100 diastolic. Her urinary output was normal, and there was no evidence of fluid retention. Despite seemingly adequate therapy, the patient did not become mentally alert nor did she take food. She went progressively downhill, and died without terminal incident on December 7, 1946, twenty years after the onset of diabetes, and three years and seven months after the onset of Addison's disease.

Necropsy Findings: (Only pertinent findings will be presented). The heart weighed 335 Gm. The myocardium was of normal thickness and no areas of fibrosis were noted.

The kidneys weighed 310 Gm. The surfaces were finely granular. On microscopic section, there was a marked increase in the interstitial connective tissue and a rather extensive atrophy of the tubules. The latter contained many hyaline casts. The glomeruli showed extensive hyalinization and occasional epithelial crescents.

The liver was normal in gross apearance, on cut section, and on microscopic study. Stains for hemosiderin gave negative results.

In the pancreas only increased fibrosis was found.

Each adrenal gland weighed less than 5 Gm. On cut section, the normal architecture was completely lost. Microscopically, the adrenal was almost entirely replaced by an acute tuberculous process, represented by scattered Langhans' giant cells and diffuse epithelioid cell formation. A few areas of typical cortical cells could be distinguished.

Pathologic diagnoses were: 1) tuberculosis of the adrenal glands, and 2) chronic glomerulonephritis.

DISCUSSION

In 18 of the cases previously reported, diabetes mellitus preceded the onset of Addison's disease in 10; the reverse occurred in only 3 instances and the onset was simultaneous in the remaining 5. However, virtually the

same clinical and laboratory picture eventually prevailed in all. Progress of the patient described here illustrates the typical modifications in the carbohydrate metabolism of the diabetic, brought about by Addison's disease. In short, diabetes mellitus is attenuated by the onset of Addison's disease, as Long and Lukens demonstrated experimentally (41).

The following changes, briefly summarized, noted in our patient and in those previously described, are: a) Because glycogeneolysis increases in diabetes mellitus and glycogen formation decreases in Addison's disease, both diseases, either separately or together, lower liver and muscle glycogen. b) Decreased utilization of glucose in the tissues is noted in diabetes mellitus. This is not true in Addison's disease and, in fact, such utilization may increase. In combination, these two effects result in a decreased utilization of glucose which is probably not as great as that found in diabetes mellitus alone. c) Although gluconeogenesis increases when the two diseases occur together, it is not enhanced to the extent that it is in uncomplicated diabetes mellitus (37).

Clinically, these basic processes are manifested by: a) decrease in blood sugar level and decrease in glycosuria with the onset of Addison's disease; b) progressive decrease in the amount of insulin necessary for adequate control of the diabetes mellitus, and increased insulin sensitivity; and c) hypoglycemic reactions at higher levels of the blood sugar.

Kendall (38) has established the relative values of the chemical fractions of the adrenal cortex related to carbohydrate metabolism. The most important of these are: a) 11-dehydro-17-hydroxycorticosterone (Compound E); b) 11-dehydrocorticosterone (Compound A); and c) corticosterone (Compound B). At present, desoxycorticosterone acetate is the only adrenal steroid commercially available. In most cases, it gives adequate control when there is a high carbohydrate intake. It has been generally assumed that desoxycorticosterone acetate has no effect upon carbohydrate metabolism in chronic adrenocortical insufficiency. However, Loeb (42) has noted that hypoglycemia appears to occur more frequently in patients treated with salt and desoxycorticosterone acetate than in untreated patients. Bloomfield (10) has conclusively demonstrated that the blood sugar (fasting) decreases in the combination of diabetes mellitus and Addison's disease from a range of 188–76 mg. per cent to a range of 74–50 mg. per cent, when the patient is receiving 5 to 25 mg. of desoxycorticosterone acetate daily. In his patient this process could be repeated at will by withdrawal and reinstitution of the synthetic hormone. The insulin requirements of Bickel's case (13) dropped from PZI 50 units to PZI 10 units at the onset of Addison's disease and, with administration of desoxycorticosterone acetate, the patient showed complete freedom from glycosuria when

no insulin was given. In a similar manner, our case illustrates a rather striking fall in blood sugar and insulin requirement with the institution of desoxycorticosterone acetate therapy. There was a marked tendency toward hypoglycemic reactions previously not present when this drug was being administered. It should be emphasized that increased insulin sensitivity is to be expected in the treatment of the two diseases when desoxycorticosterone acetate is employed.

Diminution of any magnitude in insulin requirement in diabetes mellitus is unusual, once that requirement has been established. Such a diminution should suggest possible intercurrence of Addison's disease, although similar changes may be noted with the onset of liver disease (43), hypothyroidism (44), hypopituitarism (45), and congestive heart failure in the diabetic (46).

SUMMARY

1. A case of diabetes mellitus with superimposed Addison's disease is presented. This is the nineteenth such patient reported in the literature.

2. Carbohydrate and related metabolic alterations have been discussed in reference to diabetes mellitus, chronic adrenocortical insufficiency, and the combination of these two diseases.

3. In contrast to the opinion of many authors, the administration of desoxycorticosterone acetate appears to cause definite changes in the carbohydrate metabolism.

4. In the presence of a progressively diminishing insulin requirement in a diabetic, the diagnosis of Addison's disease should be considered.

REFERENCES

1. Root, H. F.: The association of diabetes and tuberculosis, *New Eng. J. Med.* **210**: 1–13 (Jan. 4) 1934.
2. Thorn, G. W., and Clinton, M., Jr.: Metabolic changes in a patient with Addison's disease following the onset of diabetes mellitus, *J. Clin. Endocrinol.* **3**: 335–344 (June) 1943.
3. Arnett, J. H.: Addison's disease and diabetes mellitus occurring simultaneously; report of a case, *Arch. Int. Med.* **39**: 698–704 (May) 1927.
4. Levy-Simpson, S.: Addison's disease and its treatment by cortical extract, *Quart. J. Med.* **1**: 99–133 (Jan.) 1932.
5. Gowen, W. M.: Addison's disease with diabetes mellitus, *New Eng. J. Med.* **207**: 577–579 (Sept. 29) 1932.
6. Nix, N. W.: Diabetes mellitus associated with Addison's disease, *Canad. M. A. J.* **49**: 189–191 (Sept.) 1943.
7. Unverricht: Insulinempfindlichkeit und Nebenniere, *Deutsche med. Wchnschr.* **52**: 1298–1299 (July 30) 1926.
8. Rowntree, L. G., and Snell, A. M.: A Clinical study of Addison's Disease, Philadelphia, W. B. Saunders Co., 1931, pp. 93–95.
9. Rogoff, M. J.: Addison's disease following adrenal denervation in case of diabetes mellitus, *J.A.M.A.* **106**: 279–281 (Jan. 25) 1936.

10. Bloomfield, A. L.: Coincidence of diabetes mellitus and Addison's disease; effect of cortical extract on glycemia and glycosuria, *Bull. Johns Hopkins Hosp.* **65:** 456–465 (Dec.) 1939.
11. Bowen, B. D.; Koepf, G. F.; Bissell, G., and Hall, D.: Metabolic changes in co-existing diabetes mellitus and Addison's disease, *Endocrinology* **30:** S1026 (June) 1942.
12. McCullagh, E. P.: Two cases of diabetes mellitus, one with myxedema and one with Addison's disease, *Cleveland Clin. Quart.* **9:** 123–134 (July) 1942.
13. Bickel, G.: Diabète pancréatique sévère, devenu aglycosurique a l'occasion du développement d'une maladie d'Addison, *Helvet. med. acta* **12:** 281–283 (June) 1945.
14. Devitt, J. S., and Murphy, F. D.: Diabetes mellitus complicated by Addison's disease; case report with a review of the literature, *Am. J. Digest. Dis.* **14:** 164–165 (May) 1947.
15. Adler, D. K.: Atypical Addison's disease associated with diabetes mellitus, *New Eng. J. Med.* **237:** 805–808 (Nov. 27) 1947.
16. Allan, F. N.: Association of diabetes mellitus and Addison's disease, *Proc. Staff Meet., Mayo Clin.* **5:** 349 (Dec. 3) 1930.
17. Brookfield, R. W., and Corbett, H. V.: Diabetes mellitus in association with degeneration of suprarenal glands, *Brit. M. J.* **1:** 231–232 (Feb. 10) 1934.
18. Heim, W.: Diabetes mellitus and Addisonische Krankheit, *Frankfurt. Ztschr. f. Path.* **54:** 250–264 (April) 1940.
19. Sprague, R. G.; Kepler, E. J.; Keating, F. R., and Power, M. H.: Coexisting Addison's disease and diabetes mellitus; comparative effects of compound E (17-hydroxy-11-dehydrocorticosterone) and allied substances in three cases, Proc. Am. Soc. Clin. Investigation, *J. Clin. Investigation* **26:** 1198 (Nov.) 1947.
20. Rhind, E. G. G., and Wilson, A.: Diabetes mellitus in Addison's disease, *Lancet* **2:** 37–38 (July 12) 1941.
21. Soffer, L. J.: Diseases of the Adrenals, Philadelphia, Lea & Febiger, 1946, p. 111–112.
22. Lowrie, W. L.; Redfern, W. E., and Foster, D. P.: Use of globin insulin in Addison's disease associated with insulin-sensitive diabetes, *J. Clin. Endocrinol.* **8:** 325–331 (April) 1948.
23. Knowlton, A. I., and Kritzler, R. A.: The development of diabetes mellitus in Addison's disease. Case report with autopsy, *J. Clin. Endocrinol.* **9:** 36–47 (Jan.) 1949.
24. Cori, C. F., and Cori, G. T.: Carbohydrate Metabolism, *in* Annual Review of Biochemistry, Stanford University P.O., California, Annual Reviews, Inc., vol. 15, p. 210.
25. Price, W. H.; Cori, C. F., and Colowick, S. P.: Effect of anterior pituitary extract and of insulin on hexokinase reaction, *J. Biol. Chem.* **160:** 633–634 (Oct.) 1945.
26. Hechter, O.; Levine, R., and Soskin, S.: Relationship between sugar concentration and glycogenetic action of insulin on rat diaphragm in vitro, *Proc. Soc. Exper. Biol. & Med.* **46:** 390–393 (March) 1941.
27. Soskin, S., and Levine, R.: Relationship between blood sugar level and rate of sugar utilization, affecting theories of diabetes, *Am. J. Physiol.* **120:** 761–770 (Dec.) 1937.
28. Stone, F. J., and Thorn, G. W.: Protein nutrition in problems of medical interest, *J.A.M.A.* **127:** 1120–1127 (April 28) 1945.
29. Best, C. A., and Taylor, N. B.: Physiological Basis of Medical Practice, ed. 4, Baltimore, Williams and Wilkins Co., 1945, p. 574.

30. STETTEN, DEW., JR., and KLEIN, B. V.: Studies in carbohydrate metabolism; effect of hypo- and hyperinsulinism in rabbits, *J. Biol. Chem.* **162:** 377–382 (Feb.) 1946.
31. BRITTON, S. W., and COREY, E. L.: Pancreatic and cortico-adrenal involvement in carbohydrate regulation, *Am. J. Physiol.* **131:** 790–798 (Jan.) 1941.
32. HARTMAN, F. A.: Functions of the adrenal cortex, *Endocrinology* **30:** 861–869 (June) 1942.
33. EVANS, G.: Effect of adrenalectomy on carbohydrate metabolism, *Endocrinology* **29:** 731–736 (Nov.) 1941.
34. RUSSELL, J. A.: Relationship of anterior pituitary and adrenal cortex and metabolism of carbohydrate, *Am. J. Physiol.* **128:** 552–561 (Feb.) 1940.
35. LONG, C. N. H.; KATZIN, B., and FRY, E. G.: The adrenal cortex and carbohydrate metabolism, *Endocrinology* **26:** 309–344 (Feb.) 1940.
36. NELSON, N.; GRAYMAN, I., and MIRSKY, I. A.: Utilization of acetone bodies; influence of adrenalectomy, *J. Biol. Chem.* **132:** 711–715 (Feb.) 1940.
37. THORN, G. W.; KOEPF, G. F.; LEWIS, R. A., and OLSEN, E. F.: Carbohydrate metabolism in Addison's disease, *J. Clin. Investigation* **19:** 813–832 (Nov.) 1940.
38. KENDALL, E. C.: Hormones of the adrenal cortex, *Endocrinology* **30:** 853–860 (June) 1942.
39. EVANS, G.: The adrenal cortex and endogenous carbohydrate formation, *Am. J. Physiol.* **114:** 297–308 (Jan.) 1936.
40. MCKAY, E. M., and CARNE, H. O.: Influence of adrenalectomy and choline on the fat content of regenerating liver during fasting, *Proc. Soc. Exper. Biol. & Med.* **38:** 131–133 (Feb.) 1938.
41. LONG, C. N. H., and LUKENS, F. D. W.: Effect of adrenalectomy and hypophysectomy upon experimental diabetes in cat, *J. Exper. Med.* **63:** 465–490 (April) 1936.
42. LOEB, R. F.: Diseases of the Adrenals, *in* Oxford System of Medicine, New York, Oxford University Press, 1947, vol. 3, p. 804.
43. BORDLEY, J. III: Disappearance of diabetes mellitus during development of cirrhosis of liver, *Bull. Johns Hopkins Hosp.* **47:** 113–122 (Aug.) 1930.
44. WILDER, R. M.; FOSTER, R. F., and PEMBERTON, J. DEJ.; Total thyroidectomy in diabetes mellitus, *Proc. Staff Meet., Mayo Clin.* **8:** 720–724 (Nov. 29) 1933.
45. HOUSSAY, B. A., and BIASOTTI, A.: The hypophysis, carbohydrate metabolism and diabetes, *Endocrinology* **15:** 511–523 (Nov.–Dec.) 1931.
46. JOSLIN, E. P.; ROOT, H. F.; WHITE, P.; MARBLE, A., and BAILEY, C. C.: The Treatment of Diabetes Mellitus, ed. 8., Philadelphia, Lea & Febiger, 1946, p. 513–514.

CORTICOADRENAL TUMOR WITH HYPOGLYCEMIC SYNDROME, GOITER, GYNECOMASTIA AND HEPATOSPLENOMEGALY

JUAN JOSÉ STAFFIERI, M.D., OSCAR CAMES, M.D. AND JOSÉ M. CID, M.D.

From the Service of Endocrine Diseases at the Italian Hospital, British Sanatorium and Medical School of the University of the Littoral, Rosario, Argentina

THE most frequent disturbance in carbohydrate metabolism observed in cases of corticoadrenal tumor is a diminished tolerance to glucose, with hyperglycemia. It is therefore interesting to record a case of corticoadrenal tumor in which typical hypoglycemic crises occurred repeatedly, without any other sign of Addison's disease. The clinical diagnosis was hyperinsulinism, thought to be caused by a pancreatic new growth, but the large tumor removed at operation was revealed by the pathologic examination to be a corticoadrenal tumor of embryonic type. The thyroid, the liver and the spleen were enlarged, and bilateral gynecomastia was present.

A preliminary report of this case was published in December 1947 (1). Later Broster and Patterson (2) reported a case of corticoadrenal tumor in a young female with intense virilization and hypoglycemic crises; but no mention is made of enlargement of the thyroid, liver and spleen.

CASE REPORT

A. N., was a 25-year-old, single, house painter. Two months before coming to our attention, being in apparently normal health, he could not be awakened one morning. The doctor who was called found him bathed in sweat, more or less awake, and mentally confused. Sweetened orange juice was given and he rapidly recovered. A similar crisis occurred before breakfast four or five times at increasingly shorter intervals. Determinations made during the crises showed blood sugars below 30 mg. per cent, and a rapid return to normal after injecting glucose solution. The first morning in the hospital he was awakened with difficulty and was mentally confused, excited, stammering incoherently and sweating profusely; the blood sugar was 24 mg. per cent. A few minutes after the ingestion of sweetened milk he was again normal.

Physical examination revealed a diffusely enlarged and hard thyroid, without signs of inflammation. The patient had noted this enlargement four months before the date of examination. There was moderate, painful, bilateral gynecomastia. The liver was enlarged, its lower border being palpated 6 cm. below the ribs; its surface was smooth and painless on palpation. The spleen was enlarged on percussion and the lower pole could be felt on palpation. In the right flank a tumor the size of a large grapefruit with a lobulated, irregular surface, was felt. Its upper extremity could not be clearly separated from the liver; its posterior aspect was in contact with the lumbar region and it followed the respiratory movements. It appeared to be an enlarged kidney. The left kidney could

Received for publication July 31, 1948.

not be palpated. Genital development and secondary sexual characteristics, such as hair distribution, were normal.

Several hypoglycemic crises with the blood sugar between 30 and 47 mg. per cent were observed. With carbohydrate and protein-rich meals given at short intervals (not longer than three hours) these crises were prevented. Sugar tolerance tests (Exton-Rose and five-hour blood sugar curve) gave results similar to those found in hyperinsulinism (Figs. 1 and 2).

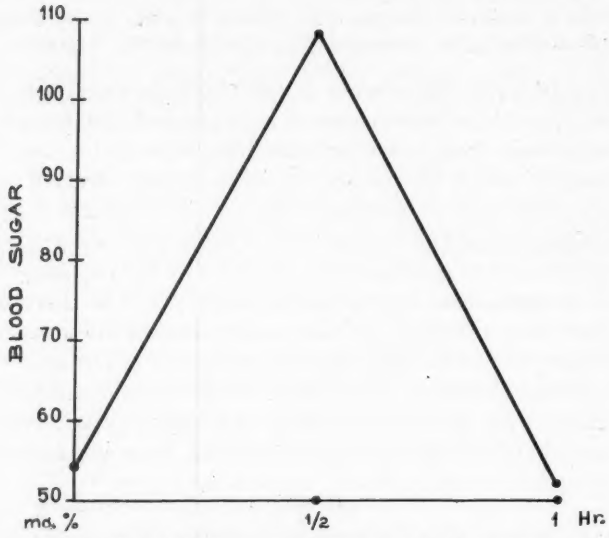


FIG. 1. Exton-Rose sugar tolerance test, before operation.

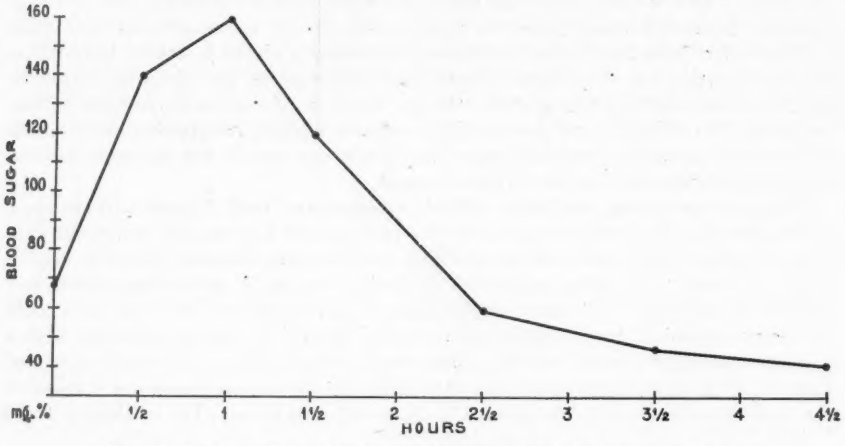


FIG. 2. Prolonged five-hour sugar tolerance test, before operation.

The urinary excretion of 17-ketosteroids was 23.24 mg. per day. The hippuric acid test (intravenous) showed an excretion of 1 gram. Blood cholesterol was 190 mg. per cent and the prothrombin test plasma clotting time was 11 seconds. The basal metabolic rate was plus 13 per cent and the electrocardiogram was normal.

FIG. 3. Section of the tumor, showing its relation to the kidney.

A complete x-ray examination was made. The sella turcica was normal. The urinary tract was explored by injection of a contrast substance. The right renal pelvis and ureter were found to be displaced towards the midline. The pelvis on each side was deformed, the calices were lengthened and abnormally separated as is seen in cases of polycystic kidney. The renal shadows were enlarged. The results of intra-ureteral injection confirmed these findings. The stomach and duodenum were found displaced towards the left and the right angle of the colon was at a subnormal level. This displacement was

caused by a tumor situated outside the gastro-intestinal tract, which was otherwise normal.

The patient was operated on under ether anesthesia. The pancreas was carefully explored and found to be macroscopically normal. No aberrant pancreas was found. The liver was enlarged and the kidneys were apparently normal. A large tumor adherent to the right kidney was removed together with the kidney (Fig. 3).

Postoperative course: The patient made a good recovery. Desoxycorticosterone acetate (10 mg.) was injected as a precaution against acute corticoadrenal insufficiency and saline and glucose were injected by the drip method.

From the third day on, the blood pressure remained stable at the normal level. The blood sugar did not fall below 100 mg. during the first forty-eight hours, and remained normal after glucose injection was discontinued on the sixth day. Hypoglycemic crises did not occur after operation.

The thyroid commenced to decrease immediately after the operation, was reduced to

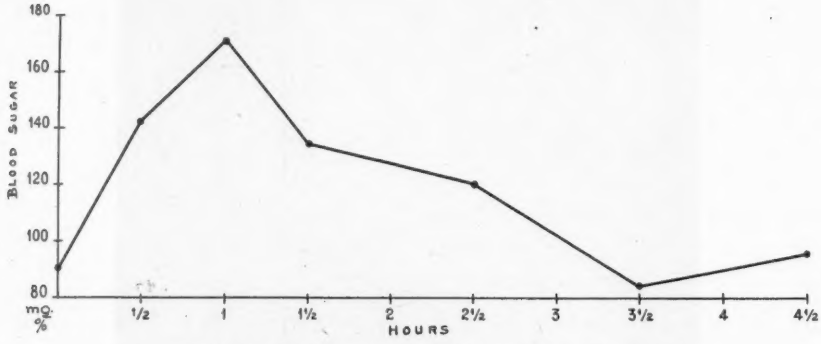


FIG. 4. Prolonged five-hour sugar-tolerance test, after operation.

half its size and was much softer by the sixth day. Gynecomastia also retrogressed; by the sixth day it had disappeared completely on the right side and was considerably reduced on the left side. Two weeks later the patient left the hospital in excellent condition.

Deep radiotherapy was given over the area previously occupied by the tumor.

Two months after leaving the hospital the patient came in for a complete examination. The thyroid was considerably reduced in size and hardness. Gynecomastia had disappeared completely. The liver and spleen remained enlarged but at a later examination performed after another interval of three months, these organs had returned to the normal size. The patient was again carefully examined every three months up to two years after the operation, during which time he has remained without relapse and in all respects is apparently normal. The sugar tolerance curve (Fig. 4) confirmed the complete recovery.

Pathologic examination: A rapid microscopic examination of the tumor made during the operation showed it consisted of malignant, atypical tissue, but did not permit the identification of its origin.

The tumor had an ovoid form, 20 ×9 ×11 cm. It formed a single mass, attached to the

kidney, but without invading the renal parenchyma. It was covered by lax, richly vascularized membranes. In section it showed a pale pink, smooth, soft, elastic surface, with small yellow opaque zones, and star-shaped areas of sclerosed tissue, distributed irregularly throughout the tumoral mass. The surface secreted abundant mucus.

Microscopic examination: "Under low power the tissue of the tumor is seen to be continuous, interspersed with numerous cavities of different size and shapes. There are also zones where the cells are more closely packed (Fig. 5). Under high power the continuity of the mesenchymatous and epithelial cells is evident (Fig. 6).

FIG. 5. Low power photomicrograph. In the center a dense zone, surrounded by lax structures. Several cavities are visible. ×100.

The tumoral parenchyma has no precise limits; it forms extensive diffuse zones, or else small rounded ones which occupy a single microscopic field. A typical feature of the tumor is the unequal distribution of the cells; in parts these are closely packed, while in others there is a lax structure with the cells widely spaced. The dense parts occupy the center and the lax ones the periphery of the neoplastic areas; there is no clear cut separation between the two and the latter are apparently transformed into the former in the course of development.

The lax structure is continuous with the connective tissue, and the cells of both intermingle. The perivascular fibrocytes in these areas, as they increase in numbers, become rounded and their nuclei are more loaded with chromatin. Thus cells are formed which still retain the fundamental features of fibroblasts and have some cytoplasmic reticulation (Fig. 7). Gradually, without any definite zone of separation, the lax areas

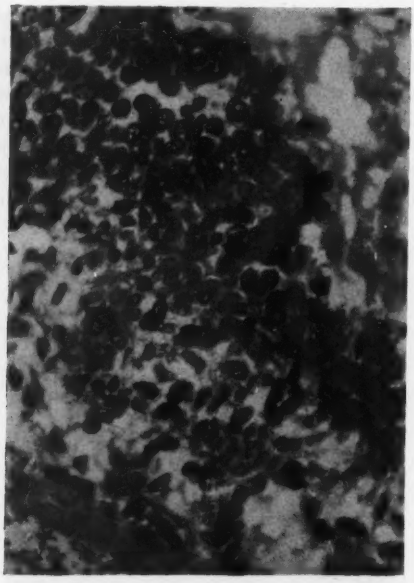


FIG. 6. Cavity formed in a dense zone. Note continuity of the cells in this zone with the lax perivascular tissue. ×400.

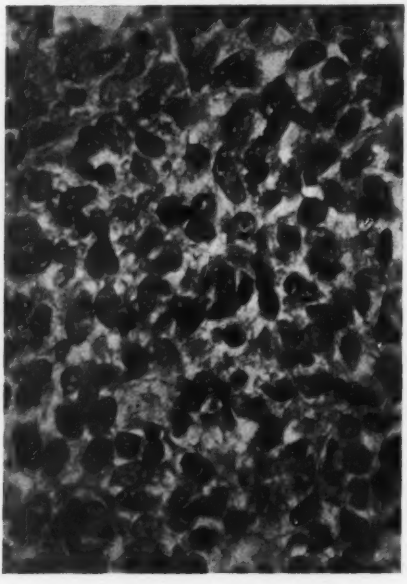


FIG. 7. Slightly reticulated sarcomatous structure. ×600.

merge into condensed areas where the cells are separated only by the clefts of sinusoids. The mesenchymatous cells are transformed into the epithelial tumoral cells. Reticulin gradually disappears as the cells take on epithelial characters (Fig. 8).

In other parts the nuclei of the tumoral cells increase in size, the cytoplasm becomes rounded and the cells are separated, appearing as spheric elements with little cytoplasm. In the center of these areas the dissociation of the cells is more marked and cavities are formed (Fig. 6).

The dense parts also show a process of apparent "xanthelasmatization" in some of the cells. There are easily recognizable accumulations of polyhedric or spheroid cells with abundant protoplasm, which is finely and evenly vacuolated. The nuclei are frequently

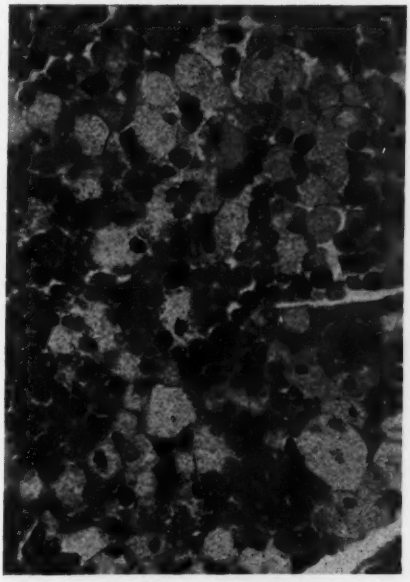


FIG. 8. Transformation of the tumor cells into spongiocytes. ×400.

pyknotic, with no nucleolus, and are sometimes situated in the center and at other times in the periphery of the cell (Fig. 8). These spongiocytes are found usually in the center of the condensed areas. They are in direct contact with a sinusoid or with the lumen of a cavity, forming part of its wall. The size of the spongiocytes varies; sometimes multinucleated cells of large size are found. The vacuolae contain lipids which stain with scarlet red.

Cavities: There are two types of cavities. Some are simple spaces in the tumoral mass without any change of structure in their vicinity; they are of different sizes and numerous enough to give a spongy aspect to the tumor. They are filled with mucus and are apparently due to chemical alterations in the collagenous substance of the stroma. Cavities of the other type are usually larger and of irregular contour. They are found isolated or in groups, and are formed in the dense parenchyma by dissociation of the cells, which

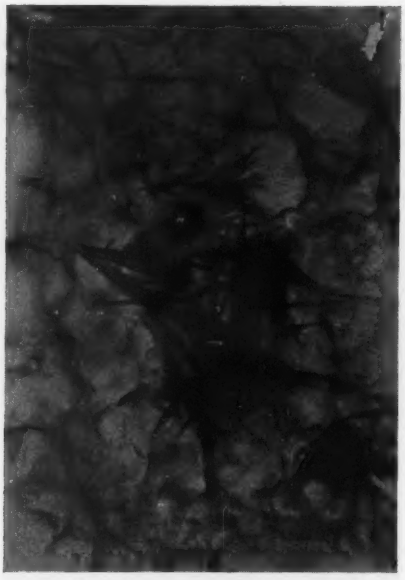


FIG. 9. Fuchsinophile crystals in a cavity. ×700.

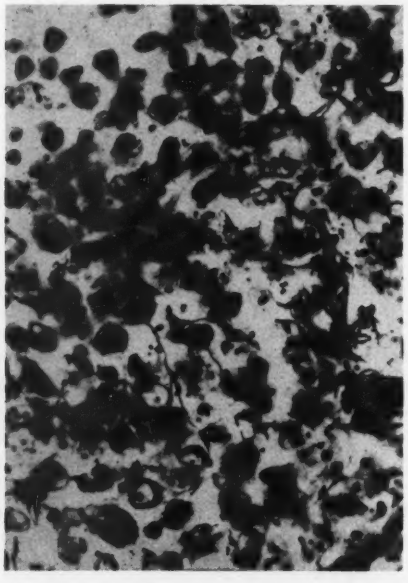


FIG. 10. Spongioplasm of the tumor cells in a dense zone (Río Ortega). ×600.

form part of the wall of the cavity and sometimes fall into it. The cavity is filled by an albuminoid substance, weakly acidophile. There are also fuchsinophile crystalloid formations within the cavity (Fig. 9).

Spongioplasm: Silver staining for reticulin shows the existence of filaments in the parenchymatous cells, specially developed in the more dense parts and in the cells of the walls of the cavities. These filaments extend into cytoplasmic processes of the cells which are not visible with other stains. This formation is the spongioplasm of the cell which increases as the cell takes on an epithelial aspect (Fig. 10).

FIG. 11. Reticulin in a dense zone (Perdrau). ×400.

Stroma: The stroma of the tumor is formed by the perivascular connective tissue, the reticulin in the dense areas, and numerous foci of sclerosis caused by involution of the parenchyma. The stroma is abundant in the lax areas; it is formed by fine bundles of collagenous fibers and a large number of reticulin fibers. In the denser parts of the tumor there are no collagenous fibers, only a fine network of reticulin (Fig. 11) which disappears in the areas of maximum condensation and epithelization (Fig. 12).

There are ordinary capillaries and sinusoids in the tumoral mass. Hyaline degeneration of the capillary walls occurs, in many places, especially near the sclerotic foci."

DISCUSSION

Ewing (3) refers to three cases of sarcoma originating in the corticoadrenal epithelium, described by Winkler, but gives no bibliographic reference. The same author quotes Rolleston and Marks' (1898) opinion that

there are sarcomas of corticoadrenal origin. Adami and Wooler (cited by Ewing) described carcinosarcomas due to cellular anaplasia provoked by local factors. We have not been able to consult the originals of these older publications, but more recent ones (1936, 1942) by Cahill and his associates (4, 5), do not report any sarcomatous tumors of the adrenal cortex.

The continuity of the parenchyma and stroma of the tumor here reported

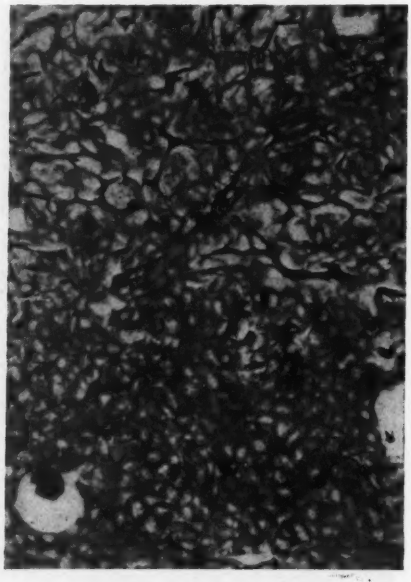


FIG. 12. In the upper half reticulin is evident, in the lower half where the cells are more densely packed there is no reticulin. ×400.

made it appear at first as a sarcoma made up of round and spindle-shaped cells. Further examination of the dense areas and the cavities in their midst showed the epithelial nature of the cells and the gradual transition of the stroma into the epithelial cells. The mesenchymatous origin was thus clearly established. The corticoadrenal nature of these cells was shown by finding what at first seemed a process of "xanthelasmatization," but was found to be really the transformation of the cells into spongiocytes loaded with lipids.

The sarcomatous nature of the tumor indicates that its origin should not be looked for in adult corticoadrenal cells, but in an embryonic tissue capable of development and differentiation. The interpretation of the histologic aspect as that of a tumor originating in adult corticoadrenal tissue

which suffered anaplastic regression to an embryonic type, cannot be accepted because of the continuity of the stroma and parenchyma, a feature which is not observed in a typical epithelial tissue. Furthermore it must be considered as a tumor of low malignancy, since it did not invade the kidney, nor produce metastases.

From the pathologic point of view the tumor in our patient can be classified as a "sarcomatous dysembryoplasia with corticoadrenal differentiation." Unfortunately it was not possible to study the hormonal content of the tumor, nor to determine the urinary excretion of estrogens which might have enlightened us as to the mechanism of the goiter and gynecomastia observed.

It is interesting to note that gynecomastia has been reported in patients treated with massive doses of insulin (6) and in cases of Addison's disease in which the patients were treated with corticoadrenal extracts, the mammary gland returning to the normal condition when treatment was discontinued (7) (8). Several cases of gynecomastia due to adrenal carcinoma have been reported and Wilkins (9) has recently added another and reviewed the literature.

With respect to the goiter observed, it is useless to speculate on the effect the enlargement of the thyroid may have had on the carbohydrate metabolism in our patient, because there were no symptoms of a disturbance in thyroid function. What effect, if any, the alteration in carbohydrate metabolism may have had on the thyroid, cannot be stated. The only reference in the literature that may be pertinent is the fact observed by Bennett, Koneff and Applegarth (10) that rats with alloxan diabetes have thyroid hypofunction, with significant reduction in the weight of the thyroid. In our patient the hypoglycemic condition may have had the opposite effect, *i.e.*, the enlargement of the thyroid; but we have not found any experimental data on the effect of hypoglycemia on thyroid weight and function to substantiate this (11–16).

However it should be borne in mind that the patient had a definite sign of feminization (gynecomastia); therefore it is possible that the androgen-estrogen equilibrium was altered, and this might have had some influence on the thyroid. Unfortunately there are contradictory data in the literature on this point (17).

Long and his associates (18) have reported that corticoadrenal extracts increased the glycogen stored in the liver, in spite of a rise in blood sugar, in fasting normal and adrenalectomized rats and mice; there was a simultaneous rise in the urinary excretion of nitrogen. Therefore it seems that the adrenal cortex can accelerate the conversion of protein into glycogen. Inhibition of glycogenolysis by corticoadrenal extracts, with subsequent

accumulation of glycogen in the liver, has been demonstrated by Seckel (19), and Corey and Britton (20).

We have no data on the glycogen content of the liver in our patient, but perhaps hepatomegaly was caused by an increase in the glycogen content, as is observed in Von Gierke's hepatic glycogenosis.

SUMMARY

A case of a young man suffering from repeated hypoglycemic crises is reported. There was also thyroid hypertrophy, gynecomastia and enlargement of the liver and spleen. A large tumor was found in the right renal fossa and was removed surgically. The hypoglycemic crises did not recur after the operation and the other disturbances rapidly disappeared.

The tumor was classified as a sarcomatous dysembryoplasia with functional differentiation, because cellular elements were found which showed the transformation of embryonic stroma cells into epithelial spongiocytes loaded with lipids.

REFERENCES

1. Staffieri, J. J.; Cames, O., and Cid, J. M.: Sindrome hipoglucémico como manifestación más destacada de un tumor corticosuprarrenal, *Medicina* **7**: 543–564, 1947.
2. Broster, L. R., and Patterson, J.: Unusual case of adrenal carcinoma, *Brit. M. J.* **1**: 781 (April 24) 1948.
3. Ewing, J.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Co., 1941.
4. Cahill, G. F.; Loeb, R. F.; Kurzrok, R.; Stout, A. P., and Smith, F. M.: Adrenal cortical tumors, *Surg., Gynec. & Obst.* **62**: 287–313, 1936.
5. Cahill, G. F.; Melicow, M. M., and Darby, H. H.: Adrenal cortical tumors; types of nonhormonal and hormonal tumors, *Surg., Gynec. & Obst.* **74**: 281–305, 1942.
6. Pico Estrada, O.: El Diagnostico de los Tumores Suprarrenales, ed. 1, Buenos Aires, El Ateneo, 1940.
7. Edwards, R. A.; Shimkin, M. B., and Shaver, J. S.: Hypertrophy of the breast due to injections of adrenal cortex in a man with Addison's disease, *J.A.M.A.* **111**: 412–414 (July 30) 1938.
8. de la Balze, F. A.: Ginecomastia, *Rev. Med. Quir. de Pat. Fem.* **21**: 309–370, 1943.
9. Wilkins, L.: A feminizing adrenal tumor causing gynecomastia in a boy of five years contrasted with a virilizing tumor in a five-year-old girl, *J. Clin. Endocrinol.* **8**: 111–132 (Feb.) 1948.
10. Bennett, L. L.; Koneff, A. A., and Applegarth, P.: Anatomical evidence of thyroid hypofunction in alloxan induced diabetes mellitus, *Endocrinology* **39**: 60 (July) 1946.
11. del Castillo, E. B.; Reforzo Membrives, J.; de la Balze, F. A., and Galli Mainini, C.: Endocrinologia Clinica, ed. 1, Buenos Aires, El Ateneo, 1944.
12. Duncan, G. G.: Diseases of Metabolism, ed. 2, Philadelphia, W. B. Saunders Co., 1947.
13. Goldzieher, M. A.; The Adrenal Glands in Health and Disease, ed. 1, Philadelphia, F. A. Davis Company, 1944.

14. Houssay, B. A.; Lewis, J. T.; Orias, O.; Braun Menendez, E.; Hug, E., and Foglia, V. G.: Fisiologia Humana, ed. 1, Buenos Aires, El Ateneo, 1946.
15. Soffer, L. J.: Adrenal Glands, ed. 1, Philadelphia, Lea & Febiger, 1946.
16. Soskin, S., and Levine, R.: Carbohydrate Metabolism: Correlation of Physiological, Biochemical and Clinical Aspects, ed. 1, Chicago, University of Chicago Press, 1946.
17. Gassner, F. X.: The effect of estrogens on the thyroid, *Endocrinology* **39**: 61 (July) 1946.
18. Long, C. N. H.; Katzin, B., and Fry, E. G.: Adrenal cortex and carbohydrate metabolism, *Endocrinology* **26**: 309–344 (Feb.) 1940.
19. Seckel, H. P. G.: The influence of various physiological substances on the glycogenolysis of surviving rat liver; influence of cortical hormone added in vitro, *Endocrinology* **26**: 97–101 (Jan.) 1940.
20. Corey, E. L., and Britton, S. W.: Glycogen levels in the isolated liver perfused with corticoadrenal extract, insulin and other preparations, *Am. J. Physiol.* **131**: 783–789 (Jan.) 1941.

THE URINARY EXCRETION OF CHORIONIC GONADOTROPIN BY HUMAN FEMALES FOLLOWING PARENTERAL ADMINISTRATION OF AQUEOUS OR BEESWAX SOLUTIONS

C. W. LLOYD, M.D., E. C. HUGHES, M.D., M. L. EVA AND J. LOBOTSKY, M.S.

From the Department of Obstetrics, Syracuse University Medical College, Syracuse, New York

THE primary function of chorionic gonadotropin in the human female would seem to be that of a luteotropic hormone. Parenteral administration of chorionic gonadotropin to normal women results in the increased function of the corpus luteum (1, 2). During the so-called "ovarian phase" of pregnancy, very high levels of this material have been found (3, 4). It is probable that this high level of chorionic gonadotropin stimulates the corpus luteum to secrete amounts of steroid hormones adequate to maintain the pregnancy.

It has been shown that many pregnant women who subsequently abort have low excretion rates of this material (3, 5) and of pregnanediol (6). It would seem that a rational method of treatment of threatened abortion would be to administer adequate amounts of chorionic gonadotropin in order to supplement the deficient placental secretion. This augmentation should stimulate further activity of the corpus luteum.

Since it has been reported that from 10,000 to 20,000 international units of chorionic gonadotropin must be given in a single injection to cause enough material to appear in the urine to produce a positive Aschheim-Zondek test (1), it is probable that large amounts of chorionic gonadotropin must be used to raise the gonadotropin level to the range found in normal pregnancy. This report concerns an attempt to determine the most effective means of maintaining a high level of circulating chorionic gonadotropin.

PROCEDURE

Chorionic gonadotropin (APL),[1] in aqueous solution was given in single intramuscular injections to 8 cyclically menstruating women. The first dose tested was always 5000 I.U. In several patients excretion following different doses was subsequently studied. Five thousand I.U. of the same materi-

Received for publication July 9, 1948.

[1] APL supplied through the courtesy of Dr. E. C. Reifenstein, Jr., of Ayerst, McKenna & Harrison.

al dissolved in 1 per cent beeswax was injected at another time to 3 of these patients and to 2 others. The duration of persistence of chorionic gonadotropin in the urine was compared following injection of the gonadotropin in these two different solutions. Excretion of gonadotropin after injection of chorionic gonadotropin in water solution was also studied in 2 postmenopausal women.

A thorough clinical study was made of each patient before injection. All had kept daily basal temperature records and endometrial biopsy specimens had been taken for several months before the administration of the gonadotropin and were repeated following the injection of this material. The endometrial pattern was not altered significantly by the single injection of this material in any of the patients. Neither of the two postmenopausal women had had any evidence of spontaneous ovarian activity for over two years.

Control urine specimens on all patients consisted of three concentrated morning specimens collected before and including the morning of injection. Following the injection, all of the urine was collected in eight-hour or twelve-hour aliquots. Each aliquot was tested for the presence of chorionic gonadotropin until several consecutive specimens had failed to contain detectable amounts of the material. The presence of chorionic gonadotropin in the urine was determined by the hyperemia response of the immature rat ovary. Details of procedures as employed in our laboratory have been published previously (5). One hyperemia unit is equivalent to approximately 3 I.U. of chorionic gonadotropin.

Twenty-four hour excretion of gonadotropin by 6 pregnant women who were threatening to abort was studied before and following injection of chorionic gonadotropin in daily doses of from 5000 to 20,000 I.U. in aqueous solution.

RESULTS

Excretion of injected chorionic gonadotropin by nonpregnant women

The excretion of gonadotropin is expressed schematically in Figure 1. An explanation of that figure is as follows:

Aqueous solution: Ten recovery experiments following a single intramuscular injection of 5000 I.U. of chorionic gonadotropin in aqueous solution were performed using 8 cyclically menstruating women as subjects. Gonadotropin was recovered in the urine of 6 of the 8 patients injected with this dose.

The average period that the material was present in the urine was 39 hours, with a range of from 0 to 80 hours. In some of the subjects, the material did not appear in the urine for twelve hours after injection, whereas

in others the material was present in the first period of collection after injection. The average time before appearance was five hours.

Patient #7, who excreted large amounts of the material following injection of 5000 I.U. on two different occasions, excreted no detectable gonadotropin after injection of 4000 I.U. Patients #1 and #2 excreted no gonado-

Excretion of Gonadotropin following One Intramuscular Injection of Chorionic Gonadotropin

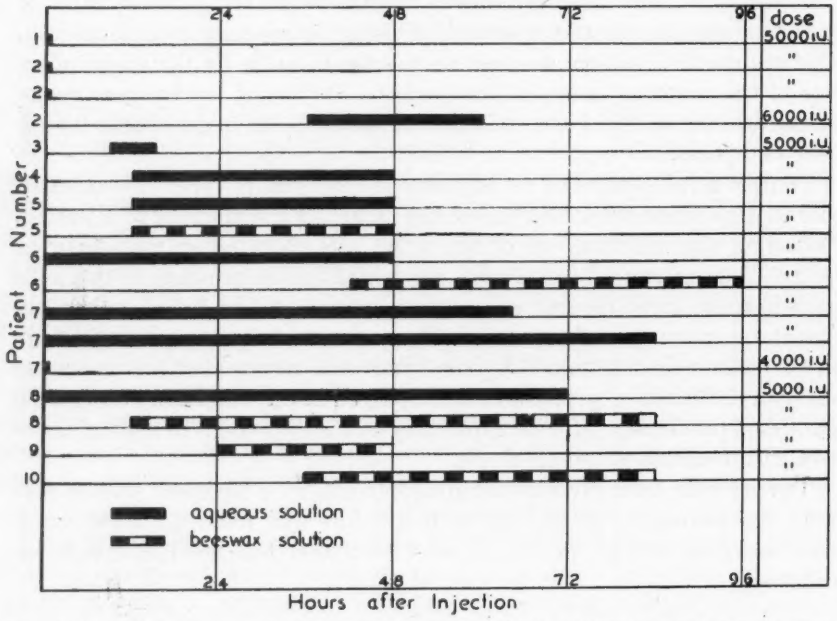


FIG. 1. Each bar represents the period during which chorionic gonadotropin was present in the urine.

tropin after receiving 5000 I.U. Patient #2 was injected at two different times with 5000 I.U. and no gonadotropin could be detected in her urine after either injection. When she received 6000 I.U. she excreted gonadotropin for 24 hours after a 36-hour latent period.

Serum from patients #1 and #4 was tested for gonadotropin. None was detectable at a level of 50 rat units per 100 cc. (2 cc. of serum injected into each of 2 rats), although in patient #4 considerable gonadotropin was present in the unconcentrated urine.

Two menopausal women, one a castrate and the other having a physiologic ovarian failure, excreted no gonadotropin after a single injection of

5000 I.U. Each patient then received an injection of 6000 I.U. Only one of them excreted gonadotropin after this injection.

Beeswax solution: Injection of 5000 I.U. of chorionic gonadotropin in beeswax was followed by its appearance in the urine of all 5 patients. There was a latent period in all varying from 12 to 42 hours before the material appeared in the urine. The average latent period was 25 hours. The material was present in the urine for an average of 47 hours, with a range of from 24 to 72 hours.

Each of the 5 patients receiving beeswax complained of local soreness. One patient (#6) developed a fever of 102°F for two days following injection of the beeswax solution.

Plasma vitamin C: Because of the probable importance of vitamin C in steroid hormone synthesis and the change of plasma vitamin C levels in cattle (7) following gonadotropin administration, plasma vitamin C levels[2] were determined on patients #1 and #4 before and after injection of chorionic gonadotropin. Before injection, average values for patient #1 were 1.26 mg. per cent and for patient #4, 1.3 mg. per cent. These values indicate that there was no deficiency of vitamin C. No change occurred following gonadotropin injection.

Excretion of injected chorionic gonadotropin by pregnant women

Chorionic gonadotropin was administered to 6 women early in pregnancy whose titers of urinary gonadotropin excretion were lower than normal and who were threatening to abort. It was found that the excretion rate could be raised to the normal range when adequate amounts of chorionic gonadotropin were given parenterally. The amount necessary to increase the excretion was usually about 10,000 I.U. daily, although in some patients 5000 I.U. daily caused a definite rise in urinary gonadotropin. Figure 2 shows the typical response of the urinary chorionic gonadotropin of a pregnant woman injected with the aqueous solution. A low excretion rate is raised to the normal range during administration of the material and then again returns to the low range when administration is stopped.

DISCUSSION

The administration of one intramuscular injection of 5000 I.U. of chorionic gonadotropin is followed in most patients by excretion of gonadotropic material in the urine for appreciable lengths of time. Those individuals receiving the material in water begin to excrete it much sooner than

---

[2] Determinations of vitamin C were performed at the suggestion of and in the laboratory of Dr. S. J. Tepperman. The method used was that of Roe and Kuether (8).

those receiving it in beeswax. The total length of time that it is present in the urine is only slightly longer following administration of the beeswax solution. It is possible that the slower rate of absorption of the beeswax-in-oil solution may result in more effective blood levels of chorionic gonadotropin, but the difficulty of estimating the blood gonadotropin renders it impractical to make direct measurements, so that this point must remain in the realm of conjecture. In our hands, the administration of relatively large amounts of the material in aqueous solution has not been accompanied by more than slight tenderness and no systemic reactions have occurred.

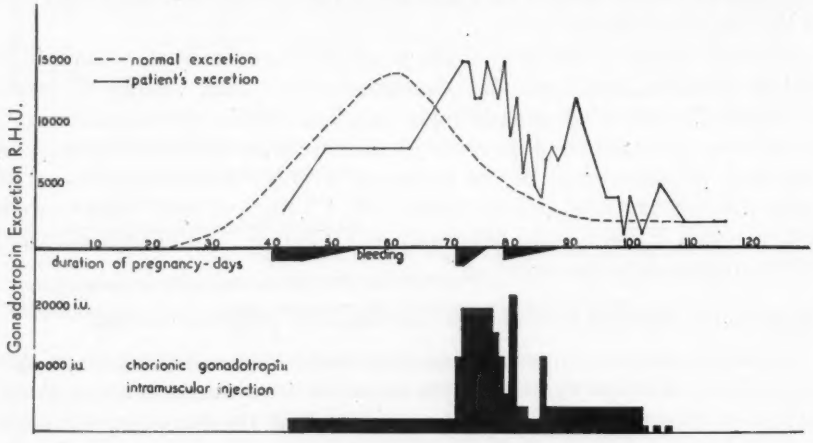


FIG. 2. Excretion of gonadotropin by a pregnant woman with a threatened abortion.

The beeswax-in-oil solution almost always causes considerable local reaction, and in one patient of the five injected, a febrile reaction lasting two days occurred.

The proportion of injected gonadotropin recoverable in the urine is considerable. For example, in one experiment gonadotropin was detectable in the urine of patient #7 for 84 hours. During this time, gonadotropic material equivalent to 530 rat hyperemia units was excreted. A rat hyperemia unit is equivalent to approximately 3 international units. Therefore, gonadotropic activity of over 1500 I.U. was recovered following the injection of 5000 units intramuscularly.

The excretion by the normal female after injection of chorionic gonadotropin of as much, if not more, gonadotropic material than is excreted by patients with inactive ovaries does not support the hypothesis advanced by Jungck, Heller and Nelson (9) that the ovaries normally inactivate gonadotropins. This does not constitute evidence against their hypothesis,

since even a vigorous inactivation of gonadotropin by the ovary probably would result in a decrease in amount of gonadotropin too small to be apparent when roughly quantitative techniques such as the method employed in this study are used.

The failure to demonstrate a change in plasma ascorbic acid following gonadotropin administration does not necessarily indicate that none of the vitamin C is utilized by the gonad in steroid synthesis. If this vitamin is employed, the amount used probably is minute compared to the supply available within the body so that no change would be detectable under the conditions of this experiment.

SUMMARY

1. A comparison has been made of the persistence of chorionic gonadotropin in the urine of normal women after injection with aqueous and with beeswax-in-oil solutions of chorionic gonadotropin.

2. A slightly longer duration of excretion of gonadotropic material was detectable following injection of the beeswax solution as compared with the aqueous solution. The beeswax solution injection was followed by a longer interval before appearance of the gonadotropin in the urine, suggesting a slower rate of absorption. Local systemic reactions were frequent following beeswax injection; no reactions followed aqueous solution injection.

3. No change in plasma vitamin C level occurred following injection of chorionic gonadotropin.

4. Excretion of gonadotropin by pregnant women can be increased by parenteral administration of chorionic gonadotropin.[3]

REFERENCES

1. Brown, W. E., and Bradbury, J. T.: A study of the physiologic action of human chorionic hormone, *Am. J. Obst. & Gynec.* **53**: 749–757 (May) 1947.
2. Browne, J. S. L.; Henry, J. S., and Venning, E. H.: Studies in corpus luteum function, *Endocrinology* **40**: 436 (June) 1947.
3. Browne, J. S. L., and Venning, E. H.: Excretion of gonadotropic substances in urine during pregnancy, *Lancet* **2**: 1507–1511 (Dec.) 1936.
4. Evans, H. M.; Kohls, C. L., and Wonder, D. H.: Gonadotropic hormone in the blood and urine of early pregnancy, *J.A.M.A.* **108**: 287, 1937.
5. Behnken, E. W.; Lloyd, C. W., and Hughes, E. C.: The ovarian hyperemia reaction: its use in qualitative and quantitative tests for urinary chorionic gonadotrophin, *Am. J. Obst. & Gynec.* In press.

[3] Since the writing of this paper, Bradbury and Brown (10) have published their findings following injection of aqueous, oil-emulsion, and beeswax preparations of chorionic gonadotropin. Using slightly different methods, they have reached essentially similar conclusions.

6. HENRY, J. S.; VENNING, E. H., and BROWNE, J. S. L.: An endocrine factor in the causation of abortion, *New International Clinics*, Series 48, **4**: 67, 1938.
7. PHILLIPS, P. H.; LARDY, H. A.; BOYER, P. D., and WERNER, G. M.: The relationship of ascorbic acid to reproduction in the cow, *J. Dairy Sc.* **24**: 153, 1941.
8. ROE, J. H., and KUETHER, C. A.: The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid, *J. Biol. Chem.* **147**: 399 (Jan.) 1943.
9. JUNGCK, E. C.; HELLER, C. G., and NELSON, W. O.: Regulation of pituitary gonadotrophic secretion: inhibition by estrogen or inactivation by the ovaries? *Proc. Soc. Exper. Biol. & Med.* **65**: 148, 1947.
10. BRADBURY, J. T., and BROWN, W. E.: Absorption and excretion of chorionic gonadotropin administered intramuscularly in women, *J. Clin. Endocrinol.* **8**: 1037–1042 (Dec.) 1948.

RESULTS OF PROLONGED MEDICAL TREATMENT OF OBESITY WITH DIET ALONE, DIET AND THYROID PREPARATIONS, AND DIET AND AMPHETAMINE*

DAVID ADLERSBERG, M.D. AND MARTIN E. MAYER, M.D.

From the Nutrition Clinic of the Medical Service of the Mount Sinai Hospital, New York, N. Y.

THIS study presents the results of the treatment of obesity under controlled conditions[1] in the Nutrition Clinic of a large general hospital during an observation period of five and one half years. It includes 299 patients—27 men, 272 women. Moderate uncomplicated hypertension without evidence of heart failure was present in about 25 per cent of the group; other forms of cardiovascular disease as well as diabetes were excluded. Each patient remained under regular observation by the same physician at intervals of one to four weeks throughout the entire period of treatment.

The patients were classified according to age, sex and degree of overweight before treatment was initiated. Class I represented those with an overweight of 1 to 20 per cent; class II, 21 to 40 per cent; class III, 41 to 60 per cent; class IV, 61 to 80 per cent; and class V, over 80 per cent. The majority of the patients were in classes II and III. Ideal weight was estimated by the usual standards of sex, age, height and weight from tables of the Life Extension Institute.

Treatment in 132 patients consisted of a low calorie diet of approximately 1200 calories, without any additional medication (group A). In 61 patients, a preliminary period of treatment with diet alone was followed by a second period during which time the same diet was combined with oral thyroid medication, usually 2 to 3 grains of desiccated thyroid daily (group B). In 90 patients a preliminary period of treatment with diet alone was followed by a second period of diet combined with oral administration of 5 to 10 mg. of amphetamine sulphate twice a day one to two hours before lunch

Received for publication July 22, 1948.

* Read by title before the Thirtieth Annual Meeting of the Association for the Study of Internal Secretions, Chicago, June 18–19, 1948.

[1] The patients included in this study were seen in the clinic at regular intervals. Their heart action, pulse rate, blood pressure and body weight were determined regularly. They were given a supply of medicine to cover the interval between visits and on their return, an inquiry was made concerning their adherence to diet and their drug intake. The procedure was, in this respect, the same for groups A, B and C.

and before dinner (group C). A final group of 16 patients was successively treated by diet alone, diet and thyroid medication, and diet and amphetamine medication (group D).

Low calorie diet without any medication (Group A)

This group consisted of 132 patients—18 men and 114 women. The age varied from 13 to 76 years, and the average age for the five weight classes ranged from 45 to 58 years. It was of interest that individuals over 70 were present only in weight classes I and II. The upper age limits in classes III

TABLE 1. TREATMENT WITH DIET ALONE (GROUP A)

Weight class	Over-weight %	Age in years		Sex		Total No. of indi-viduals	Average duration of treat-ment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)
		Range	Average	Male	Female				
I	1–20	31–76	58	9	39	48	11.7	6.2	0.5
II	21–40	22–72	53	5	38	43	10	10.4	1
III	41–60	13–65	47	4	27	31	5.5	7.8	1.2
IV	61–80	26–55	45	0	6	6	5.5	15.04	2.8
V	over 80	38–67	46	0	4	4	5.5	12.25	2.2

to V were 55 to 67 years. Although only comparatively small numbers of individuals were represented in classes IV and V, the general impression was that higher degrees of obesity, as is well known, affected longevity.

The treatment of this group consisted exclusively of a 1200 calorie diet. The comparatively high protein content of the diet (at least 100 Gm.) was stressed previously (1).

The loss of weight in the various weight ranges showed considerable fluctuation. The average monthly loss of weight was 0.5 lb. in class I, which gradually increased to 2.8 lbs. in class IV and decreased slightly to 2.2 lbs. in class V. Despite the slight discrepancy of class V, which included only 4 cases, the impression was that with higher initial overweight there was a higher average monthly loss of weight. A detailed presentation of these data is given in Table 1.

Low calorie diet in combination with thyroid medication (Group B)

This group included 61 patients—2 men and 59 women. The age varied from 15 to 63 years, and the average age for the five weight classes ranged

from 39 to 47 years (Table 2). Compared with the previous group, this one was composed of somewhat younger individuals. The age difference, which is still more pronounced in group C, is explained by the selection of patients for the various forms of treatment. Individuals of older age groups frequently presented clinical contraindications for the use of thyroid or amphetamine medication and had, therefore, to be excluded from groups B and C.

TABLE 2. PRELIMINARY TREATMENT WITH DIET ALONE AND LATER TREATMENT WITH DIET PLUS THYROID (GROUP B).

Weight class	Overweight %	Age in years		Sex		Total No. of individuals	Diet alone			Diet plus thyroid		
		Range	Average	Male	Female		Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)	Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)
I	1–20	34–56	47	0	12	12	2.2	3.1	1.3	6.1	2.3	0.4
II	21–40	15–62	43	1	14	15	3.6	4.3	1.2	5	4.4	0.9
III	41–60	19–63	46	0	21	21	3.4	4.4	1.3	5.5	3.5	0.6
IV	61–80	28–50	39	1	8	9	4.6	2.6	0.6	6.7	5.5	0.9
V	over 80	32–57	46	0	4	4	3.6	9.1	2.5	6.3	10.4	1.6

All patients of this group were treated at first by diet alone, approximately 1200 calories, for a period of 2.2 to 4.6 months. The average monthly loss of weight during this time varied from 0.6 to 2.5 lbs. The pattern of weight loss for the various weight classes, however, is different from that in group A. The average monthly weight loss in group B was almost identical for the weight classes I, II, III (1.2 to 1.3 lbs), decreased considerably in class IV (to 0.6 lb) and rose to the peak of 2.5 lbs. in class V. Because of the small number of individuals included in weight classes IV and V no definite significance can be attached to the results in the latter two classes. Individuals of weight classes I to III of group B showed a better response to diet alone than those in group A. The absence from group B of very old individuals (whose cooperation as a whole was less satisfactory than that of younger individuals) may offer an explanation for this observation. It was frequently noted that men and women over 60 had resigned themselves to their obesity and showed lack of cooperation and indifference.

The preliminary period of diet alone was followed by a second period of 5 to 6.7 months during which time the same diet was combined with oral administration of 1 to 4 grains of desiccated thyroid daily (usually 2 to

3 grains). Special attention was paid to evidences of hyperthyroidism, such as palpitation, tachycardia, and increased perspiration. On the whole, intolerance to therapy was rarely observed. The average weight loss during the diet-plus-thyroid period ranged from 0.4 to 1.6 lbs and thus was certainly less satisfactory than during the diet-alone period. Detailed data for loss of weight in the five classes are presented in Table 2.

The addition of thyroid medication under the conditions of this investigation was of no help in achieving the continued progressive weight reduction in later stages of the treatment.

Low calorie diet in combination with amphetamine medication (Group C)

This group included 90 patients—6 men and 84 women. The age varied from 15 to 64 years and the average age for the weight classes I to V ranged from 34 to 45 years (Table 3). It is evident that the individuals of this group were a few years younger than those of group B and especially those of group A. As is known, hypertension limits the use of amphetamine, and

TABLE 3. PRELIMINARY TREATMENT WITH DIET ALONE AND LATER TREATMENT WITH DIET PLUS AMPHETAMINE (GROUP C).

Weight class	Overweight %	Age in years		Sex		Total No. of individuals	Diet alone			Diet plus amphetamine		
		Range	Average	Male	Female		Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)	Average duration of treatment in months	Average total loss of weight (pounds)	Average loss of weight per month (pounds)
I	1–20	31–64	45	1	18	19	5.2	1.8	0.3	3.8	4.2	1.1
II	21–40	15–64	42	4	32	36	2.4	4.2	1.7	4.6	8.1	1.8
III	41–60	16–59	36	1	22	23	1.2	1.8	1.5	6.7	10.2	1.7
IV	61–80	25–47	38	0	7	7	2.2	4.2	1.9	2.7	3	1.1
V	over 80	20–40	34	0	5	5	1.9	1.9	1	11.75	19.6	1.7

the observation of this principle restricted the selection of patients in group C to lower age groups.

All patients of group C were treated at first with a 1200 calorie diet for 1.2 to 5.2 months. The average monthly loss of weight during this time varied from 0.3 to 1.9 lbs. Compared with the results in the diet-alone periods in group A and B the response to diet therapy alone in group C was satisfactory.

The preliminary period of diet alone was followed by a second period of 2.7 to 11.8 months during which time the same diet was combined with oral administration of 5 to 10 mg. of amphetamine sulphate twice daily, one to two hours before lunch and before dinner.

The average monthly loss of weight during the amphetamine period ranged from 1.1 to 1.8 lbs. The results are better than those achieved under similar circumstances with thyroid medication in group B. Thus, the continuation of a satisfactory reducing regimen over longer periods gave better results with amphetamine than with thyroid medication. However, a certain caution is indicated because of the different rate of weight reduction observed in the preliminary diet-alone periods in groups B and C.

Diet alone, diet and thyroid medication and diet and amphetamine medication used successively in the same individual (Group D)

This group included 16 individuals whose age ranged from 20 to 65 years. Four of them were in weight class I, 7 in weight class II, 2 in weight class III, and 3 in weight class IV. All patients were treated first by diet alone over a period of 0.75 to 10 months (average 3.8 months). In the second period desiccated thyroid was added and in the third period, amphetamine, the same quantity as used before in groups B and C. The sequence varied with the individuals, as some were first subjected to amphetamine and then to thyroid medication. The thyroid medication was administered over a period of 0.5 to 27 months (average 6.8 months) and the amphetamine medication over a period of 0.5 to 15 months (average 3.3 months). Again there were wide fluctuations, but the average figures for the four weight classes confirmed the results obtained with larger numbers of individuals in groups A to C. The average loss of weight in the diet-alone period in this group was 0.6 lb.; with the addition of thyroid, 0.1 lb.; and with the addition of amphetamine, 0.9 lb. Thus in the prolonged treatment of obesity, diet *per se* was more effective than diet plus thyroid and somewhat less effective than diet plus amphetamine.

The 'time factor' in weight reduction

Best results were obtained in the first one to two months of treatment regardless of the form of therapy used. This fact was primarily due to the greater degree of overweight at the beginning of treatment. In our opinion an additional factor was the morale of the patient which was at its best during the first one to two months of treatment. In later phases of treatment less cooperation was encountered.

It was of interest to compare the initial results obtained with diet alone, diet and thyroid, and diet and amphetamine medication with the later results of the same groups and weight classes (Table 4). In group A the average monthly weight loss during the initial period of one to two months was 2.5 to 3.8 lbs. while in the later course of four to twenty months the corresponding figures were 1 to 2.15 lbs. The results were even more striking in group B where the initial weight loss varied from 1.5 to 3.3 lbs. and

decreased later to 0.3 to 0.4 lb. Finally, in group C, the average monthly weight loss during the initial period was 3 to 3.3 lbs and dropped later to 0.7 to 2.1 lbs. Only weight classes I, II and III were used for this analysis since the majority of the individuals were included in these three weight classes.

TABLE 4. PRELIMINARY TREATMENT WITH DIET ALONE AND LATER TREATMENT WITH DIET PLUS THYROID OR DIET PLUS AMPHETAMINE.

	Group A			Group B			Group C		
	Diet alone			Diet plus thyroid			Diet plus amphetamine		
Overweight classes	I 1–20%	II 21–40%	III 41–60%	I 1–20%	II 21–40%	III 41–60%	I 1–20%	II 21–40%	III 41–60%
Average monthly weight loss in pounds during the 1st to 2nd months	2.5(9)*	3.84(11)	3.32(13)	2.62(2)	3.30(7)	1.52(9)	3.02(9)	3.0(14)	3.3(8)
Average monthly weight loss in pounds during the 4th to 20th months	1.0(22)	2.15(26)	1.8(17)	0.30(7)	0.35(6)	0.43(9)	0.68(6)	2.1(15)	1.55(10)

* The figures in parentheses represent the number of individuals.

It is thus evident that the 'time factor' played an important role in all three weight groups. The initial results were far superior to those obtained later. Diet alone compares favorably with diet and amphetamine, whereas diet and thyroid was disappointing in the later stages of treatment. It is therefore most important in studies of this type to separate the short-term effect strictly from the long-range results.

Untoward effects of thyroid and amphetamine medication

The pharmacology and toxicology of reducing drugs such as thyroid and amphetamine is widely known (2, 3, 4, 5, 6). In our own experience thyroid medication resulted only occasionally in tachycardia or palpitation. These symptoms were easily controlled by reduction of the dose or by discontinuation of medication.

In the use of amphetamine sulphate strict medical indications were observed. Nevertheless, occasionally the known side effects such as ir-

ritability, nervous tension, palpitation, headaches, insomnia and constipation compelled us to use smaller doses or to discontinue the drug.

A rather frequent complaint was dry mouth and often in association with it, halitosis. The impression was that the inhibition of salivation affected the bacterial flora of the oral cavity and thus resulted in an unpleasant, sweetish odor which was noticed by the patients and their families. One of our patients refused the drug on Saturdays and Sundays when she had social dates and dances from fear that her breath might be offensive. Other patients used chewing gum, "Sen Sen," and/or cigarettes to camouflage the unpleasant breath. It was advisable to stop medication during upper respiratory infections in order not to enhance the sensation of dryness of the mucous membranes. Better results were encountered with amphetamine in spring, fall and winter than during the hot summer months. A few of our female patients preferred not to use the drug premenstrually. In their observation the sensation of heaviness and congestion in the lower abdomen was enhanced by its use.

Much has been written recently on the etiology of obesity (7) with special emphasis on psychologic factors (8, 9, 10). The compulsive appetite is considered to be an expression of disturbed emotional life and oral gratification derived from eating represents compensation for frustration and "emotional starvation." Insecurity, professional disappointment and personality defects can be easily compensated by excessive eating. Amphetamine and its derivatives exert their effect not only by suppression of appetite but also by a special action upon the central nervous system (11).

The stimulating effects of amphetamine are noted by the patients, who occasionally will increase the dose of the drug to amounts detrimental to their health, without consulting the physician. This is done because increasingly larger doses are needed for the maintenance of the desired effects on mood and feeling of well-being. Although toxicity of amphetamine has been claimed to be minimal, poisoning by accident or uncritical use has been reported (12, 13, 14, 15, 16, 17). In our own observation two instances of amphetamine poisoning were observed. They concerned two psychoneurotic women from the private practice of one of us (D.A.) and were not included in this study. These patients refilled the prescriptions for amphetamine sulphate on many occasions without our knowledge and gradually, because of depression, raised the dose to 100 mg. per day. In both instances the results were extreme irritability and restlessness, insomnia, tachycardia and rapid respiration, The diagnosis of amphetamine poisoning was later confirmed when we learned of the patient's admission of the abuse of the drug. In both instances acute exacerbations of deeply rooted psychologic conflicts might have been additional precipitating factors for the use of toxic doses. No serious damage was encountered.

With sedatives and large amounts of fluids the symptoms of intoxication subsided in the course of twenty-four hours.

In our opinion, amphetamine preparations should not be dispensed and refilled without a physician's prescription. These drugs should not be used routinely in the treatment of obesity. Patients to whom they are administered should be closely observed (18).

The discontinuation of medication confronts many patients with rather difficult psychologic problems. Obese individuals are as a rule inclined to believe, erroneously, that overweight and fatness are caused by factors beyond their control. Even unusually intelligent and critical individuals are rarely willing to realize and/or admit the importance of overeating in their own case of fatness. A smaller group of obese individuals is fully aware of the latter factor but lack the energy and the will power necessary to adhere to restricted diets and radically to change their eating habits. A drug used in the treatment of obesity assumes the role of a fetish protecting the "unfortunate" individual against the effects of overeating. Its prolonged use, however, is loaded with psychologic hazards because it confirms the first type of patient in his false ideas, or it supplies the second with an "ersatz" morale of limited transitory value.

The discontinuation then in both types of individuals causes a vacuum and leaves them suddenly without these protective mechanisms. Increased frustration and depression often result and add a new link to the vicious circle: frustration—overeating—increased frustration—more overeating.

Observations of this sort should make a physician more than reluctant in the use of any "reducing" drug in obesity for protracted periods. Because of the peculiar psychosomatic set-up, these drugs used as fetishes against overeating prevent the average obese patient from the full realization that permanently satisfactory results can be achieved only by reducing food intake and by no other means.

SUMMARY

A group of 299 obese patients treated under controlled conditions in the Nutrition Clinic of a large general hospital showed wide fluctuations in the therapeutic results. In group A, in which only low calorie diets were employed, the average weight loss increased in proportion with the degree of obesity. Diet alone resulted in better weight reduction than diet plus thyroid medication (group B), whereas diet plus amphetamine (group C) showed somewhat better results than diet alone. In the latter group there were striking appetite-reducing effects of amphetamine sulphate with considerable loss of weight. The initial effect often faded under continued administration of the drug so that with the same dosage of amphetamine, increased food intake and weight gain resulted; to avoid this, in many in-

stances the dose of amphetamine had to be gradually raised. This is in agreement with observations from this hospital in the treatment of diabetes associated with obesity (19).

Regardless of the form of therapy employed, best results were obtained in the first one or two months of treatment. In the later stages of treatment amphetamine proved to be superior to thyroid. Nevertheless, the long-term results with diet alone compared favorably with those obtained with diet and amphetamine. It is imperative in studies on weight reduction in obesity to separate strictly the short-term effect from the long-range results. The immediate success is usually much better than the ultimate result.

The untoward effects of thyroid and amphetamine medication have been discussed. Some individuals developed addiction to amphetamine and a warning against the indiscriminate use of the drug is in order. Two instances of amphetamine poisoning were observed in psychoneurotic individuals who refilled the prescription for amphetamine sulphate on many occasions and raised the dose to toxic levels. Although no serious complications resulted in these cases, amphetamine may cause poisoning by accident or by uncritical use.

REFERENCES

1. Adlersberg, D.: The use of high protein diets in the treatment of diabetes mellitus, *Am. J. Digest. Dis.* **15:** 109 (April) 1948.
2. Rynearson, E. H., and Sprague, A. W.: Obesity, *Calif. & West. Med.* **53:** 158, 1940.
3. Shelton, E. K.: The use and abuse of thyroid, *Calif. Medicine* **67:** 9 (July) 1947.
4. Lynch, J. L.: Therapy of obesity, *Virginia M. Monthly* **74:** 362 (Aug.) 1947.
5. Graham, H. B.: Corpulence in childhood and adolescence: a clinical study, *M. J. Australia* **2:** 649, 1947.
6. Harris, S. C.; Ivy, A. C., and Searle, L. M.: The mechanism of amphetamine-induced loss of weight: a consideration of the theory of hunger and appetite, *J.A.M.A.* **134:** 1468 (Aug. 23) 1947.
7. a. Newburgh, L. H.: Nature of obesity, *J. Clin. Investigation* **8:** 197, 1930.
   b. ———: The cause of obesity, *J.A.M.A.* **97:** 1659, 1931.
   c. Evans, F. A.: Obesity: a clinical point of view, *South. Med. & Surg.* **103:** 307, 1941.
   d. Duncan, G. G.: Diseases of Metabolism, Philadelphia, W. B. Saunders Co.,1942.
   e. Sevringhaus, E. L.: Endocrine Therapy in General Practice, Chicago, Yearbook Publishers, 1947.
   f. Kunde, M. M.: The role of hormones in the treatment of obesity, *Ann. Int. Med.* **28:** 971 (May) 1948.
8. Freed, S. C.: Psychic factors in the development and treatment of obesity, *J.A.M.A.* **133:** 369 (Feb. 8) 1947.
9. Danowsky, T. S., and Winkler, A. W.: Obesity as a clinical problem, *Am. J. M. Sci.* **208:** 622, 1944.
10. a. Bruch, H.: Obesity in childhood and personality development, *Am. J. Orthopsychiat.* **11:** 467 (July) 1941.

b. WAIFE, S. O.: The pathogenesis of obesity, *Amer. Pract.* **2**: 47, 1947.
11. SOLLMAN, T.: A Manual of Pharmacology, ed. 7, Philadelphia & London, W. B. Saunders Co. 1948, p. 383.
12. ANDERSON, E. W., and SCOTT, W. C. M.: Cardiovascular effects of benzedrine, *Lancet* **2**: 1461, 1936.
13. APFELBERG, B.: Case of benzedrine sulfate poisoning, *J.A.M.A.* **110**: 575 (Feb. 19) 1938.
14. SMITH, L. C.: Collapse with death following use of amphetamine sulfate, *J.A.M.A.* **113**: 1022 (Sept. 9) 1939.
15. HERTZOG, A. J.; KARLSTROM, A. E., and BECHTEL, M. J.: Accidental amphetamine sulfate poisoning, *J.A.M.A.* **121**: 256 (Jan. 23) 1943.
16. ROSENBAUM, H. A.: Amphetamine sulphate poisoning in a child of twenty months, *J.A.M.A.* **122**: 1011 (Aug. 7) 1943.
17. WAUD, S. P.: Effects of toxic doses of benzyl methyl carbinamide (benzedrine) in man, *J.A.M.A.* **110**: 206 (Jan. 15) 1938.
18. Queries and Minor Notes: *J.A.M.A.* **137**: 756 (June 19) 1948.
19. OSSERMAN, K. E., and DOLGER, H.: The treatment of the obese diabetic. (In preparation.) Personal communication.

UTERO-TUBAL PERSUFFLATION CURVE IN MYXEDEMA. EFFECT OF THYROID THERAPY

J. C. MUSSIO FOURNIER, M.D.* AND A. POU DE SANTIAGO, M.D.

Institute of Endocrinology, Montevideo, Uruguay

IN myxedematous patients, anatomic and functional changes take place in various organs containing muscular tissue. Thus, to mention but a few of the published observations, dilatation of the heart was described by Zondek (1) in 1918, megacolon by Schippers (2) in 1929, dolicocolon by Crismer (3) in 1947, megaduodenum by Hillemand and Dugué (4) in 1947, atony of the bladder by Evans (5) in 1932. Constipation is a common finding. All these disorders disappear with the administration of desiccated thyroid.

These facts led us to investigate the condition of the muscles of the uterus and fallopian tubes in patients with myxedema. For this purpose we used persufflation of the uterus, following the utero-tubal kymographic persufflation technic as described by Rubin (6) in 1928. Our apparatus—the usual application of which is in the study of the patency of the uterine tubes—is similar to that of Rubin. It consists of a gas container from which CO_2 is supplied to the uterine cavity at the constant rate of 60 cc. per minute. The gas flows through a special cannula with an encircling rubber cone which, when pressed into the external os, ensures a gas-tight joint. A manometer is connected on a branch from the piping which carries the CO_2 to the uterus. The manometer is a U-shaped tube containing mercury and provided with a scale and a float, the movements of which are registered on a chart fixed to a drum which revolves at a peripheral rate of 4 cm. per minute. It should be borne in mind that with this manometer, a height of 1 cm. on the graph is equivalent to a CO_2 pressure of 2 cm. of mercury.

After persufflation of the uterus and fallopian tubes with CO_2, a curve is obtained showing oscillations, provided that at least one of the tubes be permeable. Three elements are to be studied in these curves, *viz.*, the height of the curve and the frequency and amplitude of oscillations. In the woman of childbearing age, the height of the curve is 6 or 7 cm., the frequency of the oscillations is 4 or 5 per minute and their amplitude, 10 to 12 mm., when obtained with the usual consumption of gas as given by Rubin. In ovarian insufficiency all three elements were found to be diminished or even absent (Rubin (7)). Rubin considered the oscillations to be due to contractions of the uterine tubes. Stabile (8, 9) does not believe that

* Director of the Institute.

the oscillations are due to contractions of the tubes, as they are obtainable in patients who have undergone bilateral salpingectomy, as long as there exists an outlet for the gas, however small, such as that remaining after puncture of the uterine wall with a needle. Stabile considers the oscillations

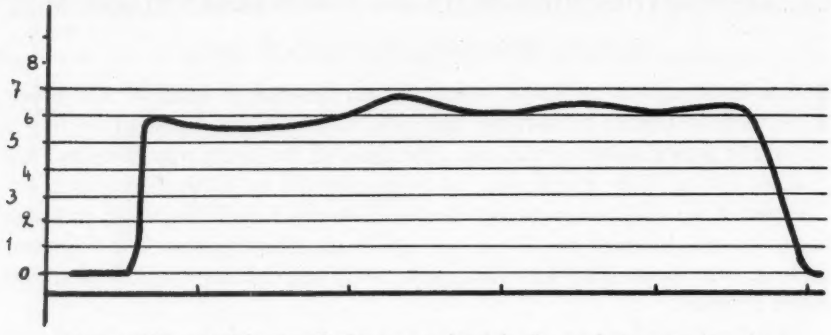


FIG. 1. Utero-tubal persufflation curve (cm.) in Case 1 before treatment.

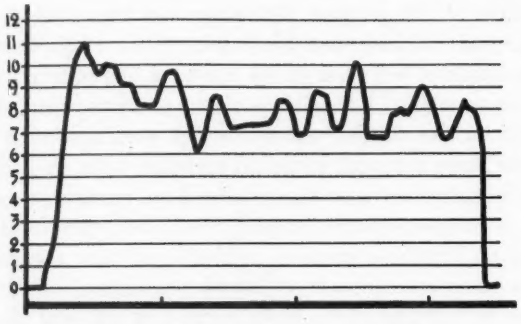


FIG. 2. Utero-tubal persufflation curve (cm.) in Case 1 after patient was treated with desiccated thyroid.

to be due to variations in the resistance of the uterus and tubes to the passage of a gas, and supposes this resistance to be directly related to the tone of the uterine and tubal muscle. Caviglia (10) confirmed Stabile's investigations. Whichever explanation be admitted—contractions of the tubes (Rubin) or variations in the tone of the uterus and tubes (Stabile)—the oscillations are evidently of muscular origin.

In this report we are publishing the kymographic persufflation curves obtained during the postmenstrual period in 7 myxedematous women before and after treatment with desiccated thyroid.

[1] The desiccated thyroid was prepared by Dispert Laboratories, Montevideo: iodine content, 0.30 Gm. per cent.

Case 1. Typical myxedema of two years' duration in a woman 45 years of age. Menstruation was regular in a 3/30 day cycle. The basal metabolic rate (B.M.R.) was minus 13 per cent. The curve shown in Figure 1 is 6 cm. high, which is below normal, and it has very few oscillations. Desiccated thyroid[1] in a daily dose of 0.05 Gm. (3/4 grain) was then given for four months. The B.M.R. rose to plus 4 per cent. A second curve was obtained (Fig. 2). The height of the curve had increased and oscillations of great amplitude and of normal frequency were observed.

FIG. 3. Utero-tubal persufflation curve (cm.) in Case 2 before treatment.

FIG. 4. Utero-tubal persufflation curve (cm.) in Case 2 after patient was treated with desiccated thyroid.

Case 2. Myxedema of two years' duration in a woman 37 years of age. Menstruation was regular. The B.M.R. was minus 26 per cent. The persufflation curve (Fig. 3) was low (between 3 and 2 cm.) but the oscillations were normal. The patient was treated with 0.10 Gm. (1½ grains) of desiccated thyroid daily for six months, with clinical improvement. The B.M.R. rose to minus 1 per cent. A new curve (Fig. 4) showed increased height, and oscillations that were normal in both frequency and amplitude.

Case 3. Typical congenital myxedema in a woman 25 years of age. Height 107 cm. Menstruation was regular in a 2/34 day cycle. The B.M.R. could not be obtained. A series of endometrial biopsies showed a complete menstrual cycle with proliferative and secretory changes. The persufflation curve was low (3 to 5 cm.) with a few oscillations of small amplitude.

Case 4. A woman 33 years of age with myxedema which began in 1935 after her fifth pregnancy. Except for amenorrhea of two months' duration, menstruation was regular in a 3/20 day cycle. The genital tract was organically normal. She showed complete alopecia, and her B.M.R. was minus 29 per cent. The persufflation curve (Fig. 5) was 7 to 8 cm. in height with no oscillations. After one month of treatment with 0.10 Gm. (1½ grains) daily of desiccated thyroid, the patient was greatly improved; the myxedema disappeared, the hair began to grow and the B.M.R. rose to minus 9 per cent. The persufflation curve was 9 to 12 cm. in height, with a few oscillations. After two years of

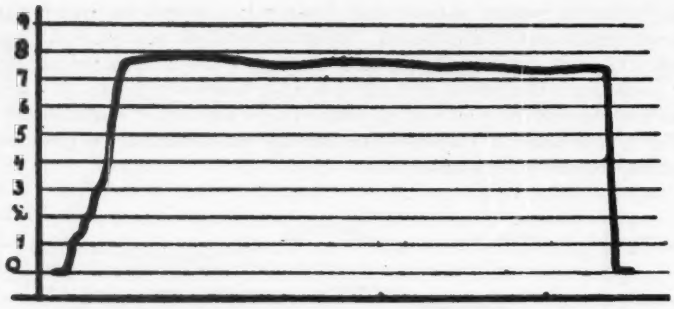


FIG. 5. Utero-tubal persufflation curve (cm.) in Case 4 before treatment.

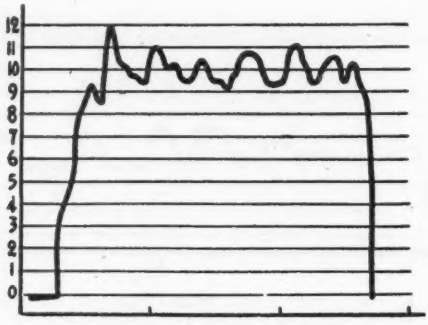


FIG. 6. Utero-tubal persufflation curve (cm.) in Case 4 after patient was treated with desiccated thyroid.

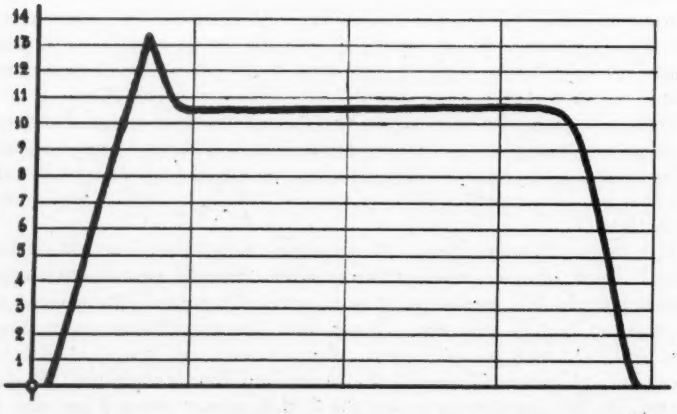


FIG. 7. Utero-tubal persufflation curve (cm.) in Case 5 before treatment.

thyroid treatment the patient was almost normal; her B.M.R. was plus 4 per cent and there were no symptoms of myxedema and no alopecia. The persufflation curve was of normal height (9 to 11 cm.) with oscillations which were normal in amplitude and frequency (Fig. 6).

Case 5. A woman, 44 years of age, in whom the diagnosis was infantile myxedema. Height 120 cm. Her voice was hoarse. Menstruation occurred in a 7/26–30 day cycle. Her B.M.R. was minus 19 per cent. A persufflation curve (Fig. 7) was normal in height (10 to 11 cm.) but showed no oscillations. Thyroid treatment for nine months with

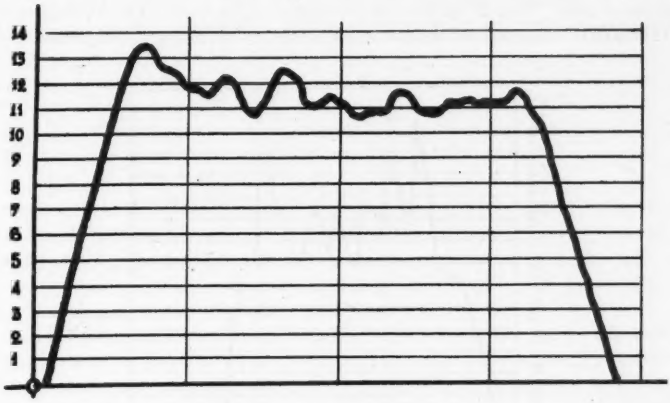


FIG. 8. Utero-tubal persufflation curve (cm.) in Case 5 after patient was treated with desiccated thyroid.

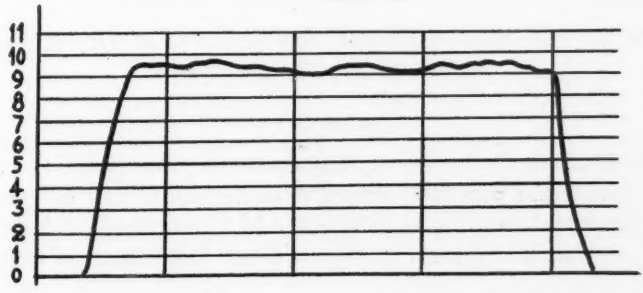


FIG. 9. Utero-tubal persufflation curve (cm.) in Case 7 before treatment.

0.05 Gm. (3/4 grain) of desiccated thyroid daily resulted in great physical and psychic improvement. The B.M.R. rose to minus 6 per cent. The persufflation curve was higher than before (11 to 13 cm.) and it showed oscillations of subnormal frequency and amplitude (Fig. 8).

Case 6. Hypothyroidism of many years' standing in a woman 32 years of age. There was an anemia with a red cell count of 2,400,000 and hemoglobin 55 per cent. The B.M.R. was minus 23 per cent. She had had four pregnancies, had normal genital organs, and

menstruated regularly in a 6/30 day cycle. The persufflation curve was normal in height but showed almost no oscillations. After five months of treatment with 0.10 Gm. (1½ grains) of desiccated thyroid daily the B.M.R. rose to minus 7 per cent. The persufflation curve showed the same height but normal oscillations.

Case 7. A woman 42 years of age. Myxedema began twenty years previously, four years after the birth of her only child. Her genital tract was organically normal and she menstruated regularly in a 7/30 day cycle. Her B.M.R. was minus 28 per cent. Figure 9 shows a persufflation curve of normal height but without oscillations. After three months of treatment with 0.05 Gm. (3/4 grain) daily of desiccated thyroid there was a general improvement. The B.M.R. rose to minus 5 per cent. The persufflation curve (Fig. 10) increased in height and showed oscillations of normal amplitude and frequency

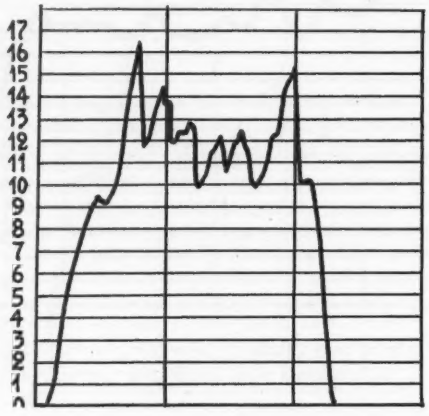


FIG. 10. Utero-tubal persufflation curve (cm.) in Case 7 after patient was treated with desiccated thyroid.

SUMMARY

A study illustrated with seven case reports is presented.

It was found that persufflation curves of the uterus and fallopian tubes in myxedematous women may be diminished in height or have oscillations which are of smaller frequency and amplitude than those of normal subjects.

Thyroid therapy produces normalization of the utero-tubal persufflation curve, coincidentally with clinical improvement and increased basal metabolism.

Whatever mechanism be accepted as explaining the elements of the kymographic curve, it is evident that a muscular factor intervenes in its pathologic modifications. This is not surprising in view of the changes which have been observed in the muscles of the heart, intestines and bladder of myxedematous patients.

REFERENCES

1. Zondek, H.: Das Mixoedemherz, *München med. Wchnschr.* **65**: 1180–1185, 1918.
2. Schippers, J. C.: Das Hirschsprungsche Syndrom als erste Erscheinung angeborenen Myxoedems; ferner einige Bemerkungen über die Ursache des Megacolon idiopathicum, *Monatschr. f. Kinderh.* **43**: 289–308, 1929.
3. Crismer, R.: La part de la thyroide dans la pathogénie du dolichocôlon, *Acta clinica Bélgica* **2**: 33–51 (Jan.–Feb.) 1947.
4. Hillemand, P., and Dugué, M.: A propos du mégaduodenum. Essai de classification et pathogénie, *Arch. d. mal. l'app. digestif.* **36**: 129–154, 1947.
5. Evans, W.: A case of myxedema with ascites and atony of the urinary bladder, *Endocrinology* **16**: 409–416 (July–Aug.) 1932.
6. Rubin, I.: Tubal patency; clinical study in 650 cases of sterility by method of per-uterine insufflation combined with kymograph, *J.A.M.A.* **90**: 99–106 (Jan. 14) 1928.
7. Rubin, I.: Influence of hormonal activity of ovaries upon character of tubal contractions as determined by uterine insufflation; clinical study, *Am. J. Obst. & Gynec.* **37**: 394–404 (March) 1939.
8. Stabile, A.: Administración de preparados hormonales e insuflación tubaria quimográfica, interpretación personal de los trabajos obtenidos, *Arch. urug. de med., cir. y especialid.* **18**: 79–91 (Feb.) 1941.
9. Stabile, A.: Las oscilaciones quimográficas de la insuflación utero-tubaria, *Arch. urug. de med., cir. y especialid.* **19**: 406–415 (Oct.) 1941.
10. Caviglia, A.: Demonstración del origen de los llamados trazados quimográficos de la trompa de la mujer, *Bol. Soc. de obst. y ginec. de Buenos Aires* **22**: 632–634 (Nov.) 1943.

The 1949 Meeting of the Association for the Study of Internal Secretions

The Thirty-First Annual Meeting of the Association for the Study of Internal Secretions will be held in the Chalfonte-Haddon Hall, Friday and Saturday, June 3 and 4, 1949, in Atlantic City, New Jersey.

We are informed by the hotel management that reservations will be difficult to secure on short notice; therefore, members are urged to make reservations at once with Chalfonte-Haddon Hall, giving time of arrival and length of stay in Atlantic City.

The scientific sessions will be held in the Viking Room, as formerly, and registration will be on the same floor. The annual dinner will be held in the Rutland Room, Friday, June 3, at 7 p.m., preceded by cocktails in the same room.

Those wishing to present papers, which will be limited to ten minutes, should send the title and four copies of an abstract of not more than 200 words, to Dr. J. S. L. Browne, Royal Victoria Hospital, Montreal 2, Canada, not later than March 1, 1949. It is imperative that the abstracts be informative and complete, with results and conclusions, in order that they may be of value for reference and suitable for printing in the program.

Nominations for the Squibb and Ciba Awards and the Ayerst, McKenna and Harrison Fellowship should be filed on special forms with the Secretary of the Association, not later than March 15, 1949, according to specifications given in the section on Awards.

Announcement of Jefferson Medical College and Hospital Fellowship

A Fellowship in obstetric and gynecologic endocrinology will be available at the Jefferson Medical College and Hospital, Philadelphia, on or about May 1, 1949, under the direction of Dr. A. E. Rakoff, Assistant Professor of Obstetrics and Gynecology, and Endocrinologist to the Department of Clinical Laboratories.

The Fellowship is available to Doctors of Medicine who have had at least one year or its equivalent of postgraduate training in obstetrics and gynecology. Applicants for the Fellowship should communicate at once with Dr. Lewis C. Scheffey, Professor of Obstetrics and Gynecology, Head of Department, and Director of Division of Gynecology, Jefferson Medical College and Hospital, Philadelphia 7, Pa.

The 1949 Meeting of the American Goiter Association

The annual meeting of the American Goiter Association will be held at the Loraine Hotel in Madison, Wisconsin, May 26 to 28, 1949. All members who have not made their hotel reservations are urged to do so immediately.

PRELIMINARY PROGRAM

Dietary Factors in the Pathogenesis of Simple Goiter.
Monte A. Greer, M.D., Martin G. Ettlinger, M.D., and E. B. Astwood, M.D., Boston, Massachusetts

Comparative Activity of Thiouracil and Other Antithyroid Compounds in the Rhesus Monkey.
D. A. McGinty, M.D., and M. L. Wilson, M.D., Detroit, Michigan

The Metabolic Fate of the Thyroid Hormone or its Derivatives.
W. T. Salter, M.D., New Haven, Connecticut

Metabolic Studies with I^{131} Labelled Thyroid Compounds.
Alexander Albert, M.D., and F. Raymond Keating, M.D., Rochester, Minnesota

The Calorigenic Properties of Tetrabromthyronine Tetrachlorthyronine as Assayed in Human Myxedema.
Jacob Lerman, M.D., Boston, Massachusetts

Thyroid Hormone-Like Properties of Tetrabromthyronine and Tetrachlorthyronine.
Charles E. Richards, M.D., Roscoe O. Brady, M.D. and Douglas S. Riggs, M.D., Boston, Massachusetts

The Antithyroxin Activity of Thyroxin Analogues.
Ruth Cortell, M.D., New York, New York

Thyroid-thyrotrophic Hormone Interaction in Body Fluids as Tested in the Starved Tadpole.
Savino A. D'Angelo, M.D., New York, New York

The Van Meter Prize Award Paper.
To be presented by winner of the Award

The Confessions of an Elderly Thyroidologist.
J. H. Means, M.D., Boston, Massachusetts

The Effects of Massive Doses of Potassium Iodide.
T. S. Danowski, M.D., Pittsburgh, Pennsylvania

The Thyroxin-like Action of Elemental Iodine
Samuel Dvoskin, M.D., New York, New York

The Functional Capacity of Various Types of Thyroid Carcinoma as Revealed by the Autoradiographic Demonstration of Radioactive Iodine.
Patrick J. Fitzgerald, M.D., New York, New York

A Method for the Preoperative Estimation of Function of Thyroid Tumors: Its Significance in Diagnosis and Treatment.
Brown M. Dobyns, M.D., and Bengt N. Skanse, M.D., Boston, Massachusetts

Thyroidectomy. (Round Table Discussion)
Richard B. Cattell, M.D., Boston, Massachusetts

Antithyroid Drugs. (Round Table Discussion)
E. B. Astwood, M.D., Boston, Massachusetts
Radioactive Iodine. (Round Table Discussion)
Mayo Soley, M.D., Iowa City, Iowa
Current Treatment of Hyperthyroidism. (Round Table Discussion)
John de J. Pemberton, M.D., Rochester, Minnesota
Treatment of Hyperthyroidism. (Round Table Discussion)
J. H. Means, M.D., Boston, Massachusetts
When is a Malignant Goiter Malignant?
Robertson Ward, M.D., San Francisco, California
Incidence of Carcinoma of the Thyroid in Nodular Goiter.
Warren Cole, M.D., Chicago, Illinois
What Thyroid Nodules Are to be Feared? A Basis for Deciding upon Surgical Exploration.
Oliver Cope, M.D., Boston, Massachusetts
Papillary Tumors of the Thyroid.
Shields Warren, M.D., Boston, Massachusetts
Non-encapsulated Sclerosing Tumor of the Thyroid.
J. Beach Hazard, M.D., and George Crile, Jr., M.D., Cleveland, Ohio
The Natural History of Thyroid Cancer.
Edgar L. Frazell, M.D., and Frank W. Foote, Jr., M.D., New York, New York
Lymphosarcoma of the Thyroid
Robert S. Dinsmore, M.D., Cleveland, Ohio
Surgical Treatment of Carcinoma of the Thyroid.
B. Marden Black, M.D., Rochester, Minnesota
Radio-Iodine Therapy of Metastatic Carcinoma of the Thyroid: A Six Year Progress Report.
S. M. Seidlin, M.D., Miss E. Oshry, I. Rossman and E. Siegel, New York, New York
Radio-Iodine in the Treatment of Metastic Cancer of the Thyroid—Credits and Debits.
Jack B. Trunnell, M.D., Miss Ruth Hill, Benedict J. Duffy, Jr., M.D., Leonidas Marinelli, M.D., Wendell Peacock and Rulon W. Rawson, New York, New York
Presidential Address—"Henry S. Plummer"
Arnold S. Jackson, M.D., Madison, Wisconsin
The Problem of Cancer of the Thyroid in a Non-Endemic Goiter Area
David H. Poer, M.D., Atlanta, Georgia
Some Goiter Problems Met With at West China Union University, Chengtu, Szechwan, China.
Charles H. Arnold, M.D., Lincoln, Nebraska
Cretinism.
Harry Colfer, M.D., Madison, Wisconsin
New Discoveries on the Innervation of the Larynx.
Brien T. King, M.D., and Ralph Gregg, M.D., Seattle, Washington
Metabolism Testing Under Anesthesia in Normal and Hyperthyroid Subjects.
Elmer C. Bartels, M.D., Boston, Massachusetts
Relationship of Lymphocytes and Fibrous Replacement to the Incidence of Postoperative Myxedema.
F. B. Whitesell, Jr., M.D., and B. Marden Black, M.D., Rochester, Minnesota
Struma Lymphomatosa.
T. C. Davison, M.D., and A. H. Letton, M.D., Atlanta, Georgia

Total Thyroidectomy: A Supplemental Report of 280 Cases of Diffuse Toxic Goiter Treated by this Method.

A. C. Scott, Jr., M.D., and P. M. Ramey, M.D., Temple, Texas

Pneumothorax Following Thyroidectomy (A Report of Two Cases)

Lindon Seed, M.D., Chicago, Illinois

A Simplified Clinical Method for the Determination of Blood Iodine.

Arthur C. Connor, M.D., Roy E. Swenson, M.D., George M. Curtis, M.D., Columbus, Ohio

Treatment of Recurrent Hyperthyroidism.

William S. Reveno, M.D., Detroit, Michigan

COURSES IN MEDICAL ILLUSTRATION

UNIVERSITY OF GEORGIA SCHOOL OF MEDICINE

The first school of medical illustration in the southeastern United States has been opened at the University of Georgia School of Medicine.

The courses are designed to equip illustrators for all types of scientific illustration, as shown in Paragraphs B1 and B2 of the following announcement. Since a knowledge of the techniques of medical illustration is necessary for the production of exemplary illustrations in other fields of scientific education and publication, special students who wish to apply such techniques to a field of classical study other than medicine may be accepted in conformity with Paragraph B1 of this announcement. Only a limited number of applicants are selected each year for this training.

Applications for admission may be addressed to the Registrar, The University of Georgia School of Medicine, Augusta, Georgia.

ART AS APPLIED TO MEDICINE*

A. COURSES FOR MEDICAL STUDENTS

1. A course in the techniques of medical illustration: open to students in each year of medical school, to postgraduates and to members of the faculty, for one afternoon a week throughout the year.

2. Research workers who require illustrations for their research will be given guidance upon request.

B. COURSES FOR MEDICAL ART STUDENTS

1. Special courses to medical illustrators who desire to take advanced work in a particular branch, or to scientific illustrators who wish to apply the techniques of medical illustration to a field of classical study other than medicine. Minimum fee, $40 per quarter.

2. A regular four-year course for beginners. This may be completed in four scholastic years, or in an accelerated course of thirty-six months. Applicants must have studied chemistry or biology, physics, zoology or

* (Extracted from the *Bulletin, The University of Georgia School of Medicine, Announcements, The Session 1948–1949*).

Antithyroid Drugs. (Round Table Discussion)
E. B. Astwood, M.D., Boston, Massachusetts
Radioactive Iodine. (Round Table Discussion)
Mayo Soley, M.D., Iowa City, Iowa
Current Treatment of Hyperthyroidism. (Round Table Discussion)
John de J. Pemberton, M.D., Rochester, Minnesota
Treatment of Hyperthyroidism. (Round Table Discussion)
J. H. Means, M.D., Boston, Massachusetts
When is a Malignant Goiter Malignant?
Robertson Ward, M.D., San Francisco, California
Incidence of Carcinoma of the Thyroid in Nodular Goiter.
Warren Cole, M.D., Chicago, Illinois
What Thyroid Nodules Are to be Feared? A Basis for Deciding upon Surgical Exploration.
Oliver Cope, M.D., Boston, Massachusetts
Papillary Tumors of the Thyroid.
Shields Warren, M.D., Boston, Massachusetts
Non-encapsulated Sclerosing Tumor of the Thyroid.
J. Beach Hazard, M.D., and George Crile, Jr., M.D., Cleveland, Ohio
The Natural History of Thyroid Cancer.
Edgar L. Frazell, M.D., and Frank W. Foote, Jr., M.D., New York, New York
Lymphosarcoma of the Thyroid
Robert S. Dinsmore, M.D., Cleveland, Ohio
Surgical Treatment of Carcinoma of the Thyroid.
B. Marden Black, M.D., Rochester, Minnesota
Radio-Iodine Therapy of Metastatic Carcinoma of the Thyroid: A Six Year Progress Report.
S. M. Seidlin, M.D., Miss E. Oshry, I. Rossman and E. Siegel, New York, New York
Radio-Iodine in the Treatment of Metastic Cancer of the Thyroid—Credits and Debits.
Jack B. Trunnell, M.D., Miss Ruth Hill, Benedict J. Duffy, Jr., M.D., Leonidas Marinelli, M.D., Wendell Peacock and Rulon W. Rawson, New York, New York
Presidential Address—"Henry S. Plummer"
Arnold S. Jackson, M.D., Madison, Wisconsin
The Problem of Cancer of the Thyroid in a Non-Endemic Goiter Area
David H. Poer, M.D., Atlanta, Georgia
Some Goiter Problems Met With at West China Union University, Chengtu, Szecnwan, China.
Charles H. Arnold, M.D., Lincoln, Nebraska
Cretinism.
Harry Colfer, M.D., Madison, Wisconsin
New Discoveries on the Innervation of the Larynx.
Brien T. King, M.D., and Ralph Gregg, M.D., Seattle, Washington
Metabolism Testing Under Anesthesia in Normal and Hyperthyroid Subjects.
Elmer C. Bartels, M.D., Boston, Massachusetts
Relationship of Lymphocytes and Fibrous Replacement to the Incidence of Postoperative Myxedema.
F. B. Whitesell, Jr., M.D., and B. Marden Black, M.D., Rochester, Minnesota
Struma Lymphomatosa.
T. C. Davison, M.D., and A. H. Letton, M.D., Atlanta, Georgia

Total Thyroidectomy: A Supplemental Report of 280 Cases of Diffuse Toxic Goiter Treated by this Method.

A. C. Scott, Jr., M.D., and P. M. Ramey, M.D., Temple, Texas

Pneumothorax Following Thyroidectomy (A Report of Two Cases)

Lindon Seed, M.D., Chicago, Illinois

A Simplified Clinical Method for the Determination of Blood Iodine.

Arthur C. Connor, M.D., Roy E. Swenson, M.D., George M. Curtis, M.D., Columbus, Ohio

Treatment of Recurrent Hyperthyroidism.

William S. Reveno, M.D., Detroit, Michigan

COURSES IN MEDICAL ILLUSTRATION

UNIVERSITY OF GEORGIA SCHOOL OF MEDICINE

The first school of medical illustration in the southeastern United States has been opened at the University of Georgia School of Medicine.

The courses are designed to equip illustrators for all types of scientific illustration, as shown in Paragraphs B1 and B2 of the following announcement. Since a knowledge of the techniques of medical illustration is necessary for the production of exemplary illustrations in other fields of scientific education and publication, special students who wish to apply such techniques to a field of classical study other than medicine may be accepted in conformity with Paragraph B1 of this announcement. Only a limited number of applicants are selected each year for this training.

Applications for admission may be addressed to the Registrar, The University of Georgia School of Medicine, Augusta, Georgia.

ART AS APPLIED TO MEDICINE*

A. COURSES FOR MEDICAL STUDENTS

1. A course in the techniques of medical illustration: open to students in each year of medical school, to postgraduates and to members of the faculty, for one afternoon a week throughout the year.

2. Research workers who require illustrations for their research will be given guidance upon request.

B. COURSES FOR MEDICAL ART STUDENTS

1. Special courses to medical illustrators who desire to take advanced work in a particular branch, or to scientific illustrators who wish to apply the techniques of medical illustration to a field of classical study other than medicine. Minimum fee, $40 per quarter.

2. A regular four-year course for beginners. This may be completed in four scholastic years, or in an accelerated course of thirty-six months. Applicants must have studied chemistry or biology, physics, zoology or

* (Extracted from the *Bulletin, The University of Georgia School of Medicine, Announcements, The Session 1948–1949*).

comparative anatomy, and the fine arts, to comprise at least ninety semester hours in a college of arts and sciences approved by the Council on Medical Education and Hospitals of the American Medical Association, and/or in a School of Fine Art approved by the Educational Division of the Veterans Administration. The School of Medicine reserves the right to require more than the minimum hours here set down for admission. Tuition and fees will be charged in conformity with tuition and fees for the School of Medicine of the University of Georgia. The course consists of the following:

(a) Surface anatomy.
(b) Anatomical dissection and drawing.
(c) The study of fresh and hardened specimens.
(d) Microanatomy and its techniques.
(e) Lettering and presentation.
(f) Design and preparation of charts and schemata.
(g) Surgical procedures and reconstructions.
(h) Techniques of medical illustration.
(i) Mechanics and ethics of medical publishing.
(j) Format and design of monographs and books.
(k) History of medical illustration.
(l) Medico-visual education and direction.

FIRST TRIMESTER

Student Registration—5 September, 1949

Jack Wilson, Associate Professor of Art as Applied to Medicine, and Director of Illustration.

Mary P. Hallinan, Student Assistant for Art as Applied to Medicine.

The 1949 Annual Meeting of the American Diabetes Association

CHALFONTE-HADDON HALL,
ATLANTIC CITY, N. J.

SATURDAY AFTERNOON, JUNE 4;
SUNDAY MORNING AND AFTERNOON, JUNE 5.

BANQUET, SATURDAY NIGHT.

Please send reservations for the banquet now to this office. Wives of members are welcome. Dinner subscription—$6.00—*Payable when you register* at the meeting.

Abstracts of
CURRENT ENDOCRINE LITERATURE

Editor: ROY HERTZ. *Collaborators:* A. R. ABARBANEL, F. N. ANDREWS, B. L. BAKER, F. A. DE LA BALZE, ISRAEL BRAM, R. A. CLEGHORN, RUCKER CLEVELAND, C. D. DAVIS, ANNA FORBES, M. B. GORDON, H. S. GUTERMAN, M. M. HOFFMAN, R. G. HOSKINS, C. D. KOCHAKIAN, H. S. KUPPERMAN, H. L. MASON, JANET W. MCARTHUR, THOMAS H. MCGAVACK, A. E. MEYER, K. E. PASCHKIS, A. B. PINTO, J. R. REFORZOMEMBRIVES, E. C. REIFENSTEIN, JR., G. G. RUDOLPH, L. T. SAMUELS

PITUITARY

BLOOM, W.: Pituitary implications in hypertrophic pulmonary osteoarthropathy, *Ann. Int. Med.* **29**: 361–370, 1948.

The case report of a patient with chronic hypertrophic pulmonary osteoarthropathy is presented. At autopsy a carcinoma of the lung with metastasis to the anterior lobe of the pituitary gland was found. The author suggests that overstimulation of the anterior pituitary by the metastatic lesion may have induced the hypertrophic osteoarthropathy. He analyzes other reports in the literature which suggest an endocrine basis for this syndrome.—*J.M.*

SCHWEITZER, FEDERICO L., and BAS, JOSÉ A.: Evaluation and differentiation of the various gonadotropic hormones by their action on the male toad, *Semana méd.* **55**: 703–708, 1948.

Various gonadotropins were assayed comparatively on immature female rats and for their effect in producing release of spermatozoa in the toad *Bufo arenarum Hensel* following the technic described by Galli Mainini. A toad unit is defined as the minimum dose that produced a positive response in two-thirds of the animals, the total number of which varied widely but was never less than 7. One toad unit of chorionic gonadotropin corresponded to 38 international units. Crude extracts from pregnant mare serum or plasma and the purified hormone gave identical results showing 1 toad unit equivalent to 150 international units. It is concluded that either the ratio of FSH to LH in the pregnant mare hormone is not altered during purification or that the toad does not respond to FSH. This latter theory is in accord with the fact that the toad is not influenced by menopause urine. The difference in reaction may be used to differentiate chorionic and pregnant mare gonadotropin.—*A.E.M.*

THYROID

BARTELS, E. C.: Propylthiouracil; its use in the preoperative treatment of severe and complicated hyperthyroidism, *West. J. Surg.* **56**: 226–235, 1948.

Propylthiouracil has been employed by the author in the preoperative treatment of 300 patients with moderate to severe hyperthyroidism. Daily doses of 200 mg. for toxic diffuse goiter and of 300 mg. for toxic nodular goiter were found to be uniformly effective.

Significant toxic reactions occurred in 2 per cent of the patients treated. These consisted of 1 febrile reaction, 4 cases of leukopenia, and 1 case of agranulocytosis. The author now combines Lugol's solution with propylthiouracil from the beginning of treatment in all patients with toxic diffuse goiter. Although the exhibition of iodine somewhat delays the return of the B.M.R. to normal, it results in more prompt subjective improvement. No death occurred after thyroidectomy in the 300 patients treated with propylthiouracil.—*J.M.*

BOWERS, J. Z.: Hyperthyroidism occurring at an early age in dissimilar twins, *Ann. Int. Med.* **29**: 935–941, 1948.

Case histories of a set of fraternal twins in whom hyperthyroidism developed at the age of 4 years 10 months and at 7 years 10 months are presented.—*J.M.*

CHAPMAN, E. M.: Treatment of Graves' disease with radioactive iodine, *West. J. Surg.* **56**: 47–51, 1948.

The history of the application of radioactive iodine to the therapy of thyroid disease is reviewed, together with certain elementary physical characteristics of radioactive iodine having a half-life of 12 hours. From experience in treating 45 patients with toxic diffuse goiter, the author concludes that the 12-hour isotope is an effective single therapeutic agent.—*J.M.*

DANZIGER, LEWIS, and KINDWALL, JOSEF A.: Thyroid therapy in some mental disorders, *Dis. Nerv. System* **9**: 231–241 (Aug.) 1948.

The authors review a considerable proportion of the relevant evidence and conclude that deficient oxidation in the brain is a potent factor in the causation of mental disorders. For the purpose of increasing the brain oxidation, thyroid substance or sodium-thyroxine was given to 5 psychoneurotic patients and to 19 psychotic patients (manic depressive and schizophrenic). Relatively large doses were employed. Recovery is reported in all the psychotic and in 2 of the psychoneurotic patients—results much better than those usually obtained in similar studies. Emphasis is laid on the desirability of prolonged treatment and of the probability that improved results might be obtained by the adjunctive use of other oxidation-promoters.—*R.G.H.*

DE ROBERTIS, E.: Proteolytic activity in the physiology, pathology and therapeutics of the thyroid gland, *West. J. Surg.* **56**: 253–269, 1948.

By means of the freezing-drying technique, the author has demonstrated the presence of intracellular colloid which is the product of secretion and reabsorption inside the cells, and has shown that the release of colloid is always intracellular. In colloid extracted from single follicles in the rat thyroid, a proteolytic enzyme which digests a gelatin substrate was found. The enzyme activity increases after injection of TSH and after the administration of iodide for a short time. After treatment with iodide for a longer time, enzyme activity becomes subnormal. From these facts, the author postulates a theory of the enzymatic reabsorption of the colloid. He suggests that the process of release from thyroglobulin from the follicle involves an enzymatic mechanism which breaks down the large protein molecule and makes possible absorption by the cells. The rate of proteolytic activity of normal and pathologic human thyroid tissue was measured. In severe toxic goiter the proteolytic activity was found to be 96 per cent above that of the normal

gland. In two cases of toxic adenoma, a difference of approximately 100 per cent was found between the proteolytic activity of the adenoma and that of the paranodular tissue. In colloid goiters the proteolytic activity was only 27.9 per cent of normal. It is suggested that the therapeutic action of iodine may depend, in part, upon its capacity to inhibit the proteolytic system. Thyroid inhibitors of the thiouracil type appear to act on special oxidative mechanisms of the cell, rather than through the proteolytic system.—*J.M.*

HEINEMANN, M.; JOHNSON, C. E., and MAN, E. B.: Serum precipitable iodine concentrations during pregnancy, *J. Clin. Investigation* **27**: 91–97 (Jan.) 1948.

Serum precipitable iodine determinations were made in 43 pregnant women and were followed after delivery in 11. Of these 43 women, ranging in age from 21 to 44 years, 29 were normal, with no personal histories suggestive of metabolic disease. In this group, concentrations of serum precipitable iodine ranged between 6.2 and 11.2 gamma per cent, the majority being a high normal or above the upper limit of the normal range of nonpregnant women (8.0 gamma per cent). Elevated values were observed as early as three to six weeks after conception, did not increase during the subsequent course of pregnancy, and subsided soon after delivery. The 10 women who aborted or threatened to abort showed concentrations (2.8 to 5.8 gamma per cent) of serum precipitable iodine which were low for normal pregnancy. In cases of threatened abortion, the serum iodine concentrations increased when desiccated thyroid was administered, but remained in the lower range of values of normal pregnancy in some instances. In one hypothyroid patient, in whom an abortion seemed inevitable in the fourth month, values for serum precipitable iodine increased from 6.6 to 10.0 gamma per cent after administration of intravenous thyroxine for two days and an increased dosage of desiccated thyroid during the remainder of pregnancy resulted in an average value of 7.8 gamma per 100 cc; a normal infant was delivered at term. Higher concentrations of serum precipitable iodine than in normal pregnancy occurred in the four hyperthyroid women; in all of these, pregnancy was maintained and the hyperthyroidism controlled by Lugol's solution alone or in conjunction with thiourea.—*T.H.McG.*

HERTZ, J.: Studies on thyrotoxicosis, *West. J. Surg.* **56**: 209–225, 1948.

Follow-up examinations were performed one to three years after thyroidectomy in 90 of 94 patients operated upon for thyrotoxicosis. Of 81 patients designated as immediately recovered, 93.5 per cent still were recorded as recovered; 2.6 per cent presented myxedema; 2.6 per cent, recurrent thyrotoxicosis; and 1.3 per cent, postoperative tetany. Of 7 patients discharged with hypothyroidism, 85.7 per cent had now recovered, whereas 14.3 per cent had myxedema. Of 5 patients discharged with persistent hyperthyroidism, 80 per cent had recovered and 20 per cent showed continuing hyperthyroidism. The significance of these findings is discussed.—*J.M.*

MCARTHUR, J. W.; RAWSON, R. W.; FLUHARTY, R. G., and MEANS, J. H.: The urinary excretion of radioactive iodine as an aid in the diagnosis of hyperthyroidism, *Ann. Int. Med.* **29**: 229–237, 1948.

The mean urinary excretion of radioactive iodine by 30 nonthyrotoxic patients was 60 per cent and by 22 thyrotoxic patients, 25 per cent. There was considerable overlap-

ping in the radioactive iodine excretion between thyrotoxic and nonthyrotoxic individuals in the range 20 to 40 per cent. The finding of a low urinary excretion of radioactive iodine aided in establishing the diagnosis of Graves' disease in certain clinically equivocal cases. The finding of a high excretion assisted in excluding the diagnosis of Graves' disease in such conditions as alcoholism, anxiety, hypertensive cardiovascular disease, Parkinsonism, pheochromocytoma and thyrotoxicosis factitia.—*J.M.*

MEANS, J. H.: Present day trends in thyroid research, *West. J. Surg.* **56**: 65–71, 1948.

The trends in twentieth-century thyroid research are reviewed, indicating the fact that the types of research undertaken have become increasingly fundamental. Another tendency which is discernible is the application of an increasing number of disciplines to thyroid problems. Among the lines which the author regards as especially promising for future research are the phylogenetic development of the thyroid, the chemical nature of TSH, the mode of action of the thyroid hormone on its end organ, and the pathogenesis of the ophthalmopathy of Graves' disease.—J.M.

THOMPSON, W. O.; THOMPSON, P. K., and MANDERNACH, D. M.: Further observations on thiouracil and related substances in the treatment of toxic goiter, *West. J. Surg.* **56**: 270–277, 1948.

The authors believe that prolonged medical control of thyrotoxicosis with the thiouracils should be attempted in only 75 to 80 per cent of patients. This form of treatment is precluded in the remainder because of toxic reactions, size of the goiter and inability to control the disease adequately. However, from their data, it appears that the prolonged administration of the thiouracils can induce a remission in approximately 60 per cent of patients with thyrotoxicosis.—*J.M.*

WILLIAMS, R. H.: An evaluation of newer methods of treatment of thyrotoxicosis, *West. J. Surg.* **56**: 72–76, 1948.

The advantages and disadvantages of treatment of thyrotoxicosis with surgery, radioactive iodine and the thiouracils are reviewed. The treatment to be employed in a given case must be selected after careful evaluation of all the factors involved. Surgery is the best established form of treatment and the long-term results are the most predictable. It is the method of choice in cases in which the goiters are very large, particularly if they are nodular, and in cases in which there is a question of carcinoma. Factors to be considered against thyroidectomy are the discomfort and expense it entails, and the serious nature of the complications. Radioactive iodine therapy is extremely valuable in certain complicated cases of thyrotoxicosis, especially when toxic reactions or refractoriness to thiouracil develop. However, the harmful effects which it may ultimately produce cannot yet be estimated. The thiouracils produce a remission in more than 50 per cent of patients treated continuously for a year. If propylthiouracil be employed, toxic reactions are relatively infrequent. The author concludes that none of these forms of therapy is ideal.—*J.M.*